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LITTER SERIATION AND THE INVASION OF FIBROSARCOMAS IN MICE*

LEONELL C. STRONG

Litter seriation has been shown to influence the time (the latent period) at which a fibrosarcoma appears in mice following the subcutaneous injection of methylcholanthrene.[1] This new principle is a variable one which apparently increases or decreases (in mice of certain strains) in a mother's body with advancing age and is handed down to her progeny by some means other than through the germ plasm (the mechanism of genes). This observation has been made on female mice which have not been injected with methylcholanthrene nor subjected to any other experimental procedure. They were mated to their normal litter-mate brothers. In the succeeding litters of mice of some strains, the average latent period for the appearance of chemically induced fibrosarcomas was gradually decreased and reached the lowest value in mice of the 7th and 8th litters. This increase of susceptibility to fibrosarcomas in successive litters is also variable in a second manner being more pronounced in mice of some strains (F_1, Brs $\times$ C_{57} and Prunt) than in other strains (2 Prunt). The reverse relationship of litter seriation has recently been found in mice of the Pridil subline where the average latent period for the appearance of fibrosarcomas is increased in mice of the successive litters.

It has also been shown that litter seriation influences the length of time that a mouse will live with the growth of a fibrosarcoma (the survival time).[2] Thus if a mouse developed a fibrosarcoma with a latent period of less than 100 days and belonged to a first litter, it lived, on an average, 52 days. The survival time gradually increased in mice of the successive litters until if it belonged to an 8th litter, it lived, on an average, 130 days. The average latent period for mice of the first four litters was 75.5 days, whereas mice of the 5-8 litters lived, on an average, 106.3 days with the growth of the same type of induced tumor. There is also an increasing sex differential as measured by the appearance of fibrosarcomas in mice belonging to the successive litters of the same parents. This sex difference in

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tumor susceptibility for mice with latent periods of less than 100 days is 13.2% in mice of the 1-2 litters, 20.9% in mice of the 3-4 litters, 23.5% in mice of the 5-6 litters and 50% in mice of the 7-8 litters.[8]

In this paper data are presented which indicate that the percentage of mice showing fibrosarcomas which invade or metastasize into the abdomen is also under the control of a principle influenced by litter seriation. By invasion is meant the appearance of discrete nodules, similar in histological appearance to the original local tumor, in the abdominal cavity. These secondary nodules usually involve the liver, kidney, or intestinal mesentery, but may also involve other viscera.

At present 788 mice of the combined Prunt and 2 Prunt sublines of the NHO strain having been injected with 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil at 60-70 days of age developed fibrosarcomas at the site of the injection of the carcinogen and have died showing the presence of the induced tumor. In other words, all mice which have died without developing a tumor or in which a chemically induced tumor has regressed are excluded from the present analysis. Of this total, 449 have been females and 339 have been males. The data obtained on mice of the different litters are presented in Table 1 and graphically in Chart 1.

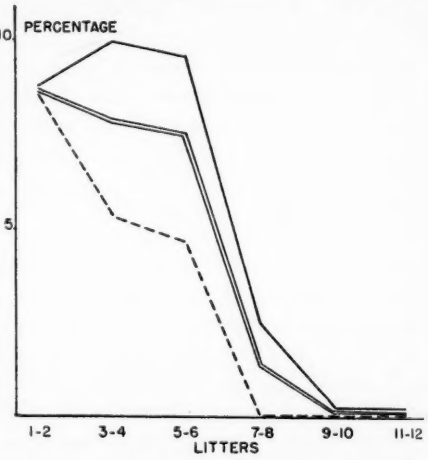

CHART 1. This chart presents the data on the percentage of mice showing invasive fibrosarcomas following the subcutaneous injection of methylcholanthrene. Litters are plotted along the base line and percentage of invasive tumors on the vertical line. Data for females on single solid line; for males on the short dash line and for combined males and females on the solid double line.

By an analysis of these data it will be seen that the maximum percentage of invasive tumors is obtained in males belonging to the first and second litters (8.53%) and in females of the third and fourth litters (10.0%). This percentage of invasive tumors decreases for both sexes in mice of the succeeding litters and reaches 0% in all mice of the advanced litters. There is a slight sex differential in the percentage of mice showing invasive

tumors between the 3-8 litters but this difference is not statistically significant (8.90% in females : 4.38% in males giving a sex difference of $4.52 \pm 1.97\%$ or $2.29 \times \sigma$). By combining data for mice of the 1-6 litters

TABLE 1. SUMMARY OF DEVELOPMENT OF INVASIVE TUMORS IN MICE

Showing: stock, litter, total number of tumors in females and males; number of mice showing invasive tumors, females and males combined and separate; and the percentage of mice (both females and males) which developed invasive tumors.

		No. tumors			*Invasion*			*% Invasion*		
Stock	*Litter*	T	♀	♂	T	♀	♂	T	♀	♂
Prunt	1-2	99	53	46	9	4	5			
2 Prunt	1-2	87	51	36	7	5	2			
Total	1-2	186	104	82	16	9	7	8.60	8.65	8.53
Prunt	3-4	151	84	67	11	8	3			
2 Prunt	3-4	165	96	69	14	10	4			
Total	3-4	316	180	136	25	18	7	7.91	10.00	5.14
Prunt	5-6	103	63	40	6	6	0			
2 Prunt	5-6	98	52	46	9	5	4			
Total	5-6	201	115	86	15	11	4	7.46	9.57	4.65
Prunt	7-8	27	16	11	0	0	0			
2 Prunt	7-8	44	26	18	1	1	0			
Total	7-8	71	42	29	0	0	0	1.41	2.38	0
Prunt	9-10	4	2	2	0	0	0	0	0	0
2 Prunt	9-10	7	3	4	0	0	0	0	0	0
Total	9-10	11	5	6	0	0	0	0	0	0
Prunt	11-12	3	3	0	0	0	0	0	0	0
2 Prunt	11-12	0	0	0	0	0	0	0	0	0
Total	3-8	588	337	251	41	30	11	6.97	8.90	4.38
Total	1-6	703	399	304	56	38	18	7.97	9.52	5.93
Total	7-12	85	50	35	1	1	0	1.17	2.00	0
Total	1-12	788	449	339	57	39	18	7.23	8.69	5.31

and comparing them with similar data for mice of the 7-12 litters, it will be seen that there is a significant difference between the groups in relation to mice which have developed invasive tumors. The x^2 value of the differ-

ence is 5.209 with a P value $.05 > P > .02$; x^2 value of this difference, with Yates' correction, is 4.247 with a P value $.05 > P > .02$. The determination of the standard deviation and the probable error also disclose a significant difference between the data obtained on mice of the 1-6 and the 7-12 litters. These values are for 1-6 litters, 647 non-invasive tumors to 56 invasive tumors or $7.97 \pm 1.02\%$ invasive tumors; for mice of the 7-12 litters these data are 84 non-invasive tumors to 1 invasive tumor or $1.17 \pm 1.16\%$ invasive tumors. The difference between the data of the 1-6 litters and 7-12 litters is $6.80 \pm 1.54\%$ invasive tumors or $4.41 \times \sigma$.

Discussion

In this paper the characteristic of invasiveness of chemically induced fibrosarcomas is shown to be associated with litter seriation. The percentage of mice showing invasive tumors gradually diminished in mice of successive litters. This means that the invasive characteristic of the malignant tumor is not exclusively determined by the tumor itself. The host somehow or other under the influence of a non-genetic principle derived from its ancestry is capable also of determining certain aspects of malignancy (e.g. the ability to invade into the abdomen).

The present investigation on litter seriation in relation to chemically induced fibrosarcomas is faced with a paradox. Sufficient data are at hand to arrive at the tentative conclusion that while the tumors in mice of the successive litters are coming earlier and earlier (a diminishing latent period), the survival time is gradually increasing and the percentage of mice showing invasive tumors is gradually diminishing. The longer survival time and the less frequent invasive tumors may be the result of the same mechanism but how this is correlated with the principle of earlier appearing tumors is not at present clear.

The small percentage of mice showing invasive fibrosarcomas (7.23% for all mice between 1-12 litters) is unquestionably the result of selection toward resistance to all types of chemically induced tumors to which the ancestry of these (Prunt and 2 Prunt) mice have been subjected for 17-20 inbred generations. Mice of all the original fifteen inbred strains which have been tested with the same amount of methylcholanthrene have all shown a considerably higher incidence of invasive tumors and the survival time of mice growing fibrosarcomas is considerably less than it is for the mice belonging to the Prunt and the 2 Prunt sublines.

Conclusions

1. The characteristic of invasiveness of chemically induced fibrosarcomas is partially determined by some new principle found in the host which has been derived from its ancestry and is differentially determined by litter seriation.

2. The number of mice developing chemically induced fibrosarcomas that have the capacity to invade the abdomen is gradually reduced in successive litters of the same breeding parents.

References

1 Strong, L. C.: A new influence on chemically induced sarcomas. Science, 1948, *108*, 688.

2 Strong, L. C.: The control of survival time in mice bearing methylcholanthrene-induced fibrosarcomas. Science (in press).

3 Strong, L. C.: A sex differential for chemically induced fibrosarcomas associated with litter seriation (in press).

ELECTRONIC BRIDGE FOR USE WITH THE GLASS ELECTRODE*

DAVID I. HITCHCOCK AND ALEXANDER MAURO†

This apparatus extends the sensitivity of an ordinary potentiometer and galvanometer so that accurate measurements can be made of the electromotive force of a cell containing a glass electrode having a resistance of about 100 megohms. The electronic bridge serves the same purpose as other circuits which have been described;[2] its advantages are good stability, high sensitivity, and moderate cost.

The bridge circuit, shown in Figure 1, is based on the use of two 959 "acorn" pentodes, as in a circuit described by Burr and Mauro.[1] These tubes are employed as "space charge grid" pentodes to reduce the grid current to less than 10^{-13} amperes;[4] one tube is used actively, the other passively. The resistance arms of the bridge consist of two fixed resistors of precision type, 2,500 ohms each, supplemented by a variable component consisting of a 20,000 ohm Helipot unit shunted by a 5,000 ohm resistor. In order to ensure maximum stability, only wire-wound resistors are used. The zero setting of the galvanometer is adjusted by the Helipot.

Experience with various insulating materials demonstrated the superiority of Lucite, which was therefore employed for the socket bases and the input switch (SW in Fig. 1). Each Lucite socket was constructed by using clips removed from a standard ceramic socket. The input switch is described in detail in Figure 2. The spring leaves were adjusted so that contact B is made before contact A is broken in order to minimize the sudden deflection or kick of the galvanometer which occurs when the circuit is opened even for an instant. The switch is operated by deflecting the center arm Y in a horizontal plane by means of a Lucite rod extending through the front panel of the case. The switch is wired so that contact A is grounded, contact B is connected to the glass electrode, and the center arm is connected to grid No. 3 of the active tube. Since the potential differences from the glass electrode cell and the potentiometer are opposed, only the difference

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between them is applied between grid No. 3 of the active tube and ground. A null measurement is made by adjusting the potentiometer dials until the galvanometer returns to its zero position.

Power for the bridge circuit is obtained from three miniature 2-volt storage cells (Willard Type BB-206-U). A 1.5-volt heavy duty dry cell is used to establish bias on the space charge grids. The vents of the storage cells were connected by plastic tubing to holes in the back of the case in order to avoid possible contamination of insulating surfaces by vapors from the electrolyte in the cells. A terminal block, not shown in Figure 1, was provided to permit recharging of the storage cells in series. This block was

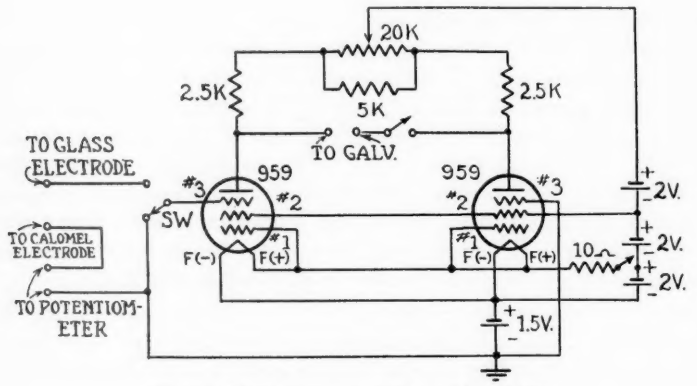

FIG. 1. Circuit diagram of electronic bridge.

made accessible only after removal of the cover of the case, in order to remind the operator to check the level of electrolyte in the cells.

The tubes, resistors, and batteries are enclosed in a metal case or housing, which serves as an electrical shield and is grounded to a water pipe. The glass electrode cell is also enclosed in a shielded box, and the leads from the bridge to the cell extend through brass tubes into this box. A telephone jack connects the bridge with wires leading to the potentiometer, where contact is made with the proper binding posts to cut out the tapping keys. Another jack connects the bridge with wires leading to the galvanometer by way of a double-pole, double-throw switch, which makes it possible to use the same galvanometer in adjusting the current in the potentiometer circuit and in making measurements by means of the bridge circuit.

The glass electrode cell consists of a glass electrode of the bulb type

(Beckman No. 1190), mounted in a water-jacketed vessel with a three-way stopcock, as described by Hitchcock and Taylor,[3] and a saturated potassium chloride calomel electrode, also in a water-jacketed vessel. The temperature of the cell is controlled within ± 0.05° C. by water flowing through the jackets from a large constant temperature bath with grounded copper walls.

Results of high precision were obtained when the bridge was used in conjunction with a high grade potentiometer (Leeds and Northrup Type K) and a galvanometer of the enclosed lamp and scale type (L. and N. No.

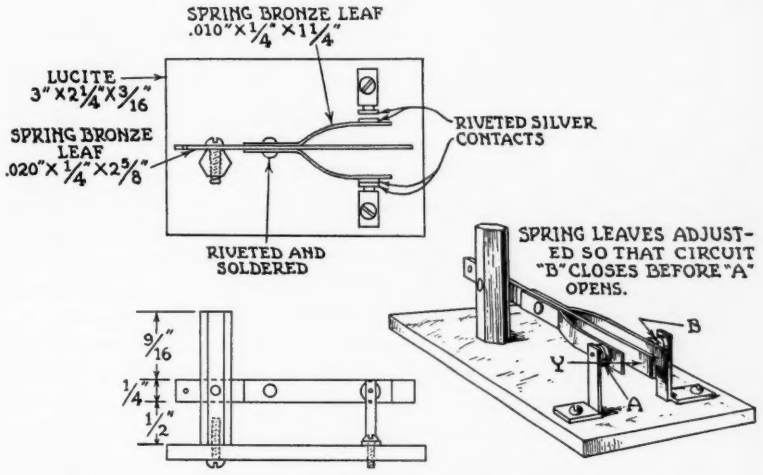

FIG. 2. Input switch for electronic bridge.

2420-c, sensitivity 0.021 microampere per mm.). With this combination it was found that 0.1 millivolt produced a deflection of 0.2 mm., which could easily be detected with the aid of a magnifying lens. Glass electrode measurements constant to 0.1 mv. were obtained within five minutes after the cell at 25° had been filled with solution at room temperature (20° to 30° C.). The accuracy with which this apparatus provides a measure of pH is indicated by the comparison with hydrogen electrode data given in Table 1. Since the figures in the last column of the table are constant within 0.3 mv., the data show that the potential of this glass electrode was affected by pH, over the range from pH 1 to 9, in the same way as that of the

hydrogen electrode, with a maximum deviation which corresponds to 0.005 in pH.

Determinations of pH differences with no uncertainty in the second decimal place have also been made by using the same electrode system, bridge, and galvanometer in conjunction with a less expensive potentiometer graduated in whole or half millivolts (L. and N. Hydrogen Ion or Students' Potentiometer).

TABLE 1

COMPARISON OF GLASS ELECTRODE MEASUREMENTS, MADE BY MEANS OF THE ELECTRONIC BRIDGE, WITH HYDROGEN ELECTRODE MEASUREMENTS AT 25° C.

Solution	pH*	E_G	E_H*	$E_G + E_H$
0.1 M HCl	1.085	0.4540	0.3083	0.7623
0.01 M HCl, 0.09 M NaCl	2.058	.3964	.3658	.7622
0.05 M KH phthalate	4.008	.2811	.4811	.7622
0.1 M acetic acid, 0.1 M Na acetate	4.648	.2433	.5190	.7623
0.025 M KH_2PO_4, 0.025 M Na_2HPO_4	6.857	.1124	.6497	.7621
0.05 M $Na_2B_4O_7$	9.180	—.0251	.7871	.7620

E_G and E_H are the electromotive forces in volts of the cells:

Hg, HgCl, KCl (satd.), solution, glass electrode.

H_2, solution, KCl (satd.), HgCl, Hg (+).

* Hitchcock and Taylor.[3]

Summary

An electronic bridge for use in glass electrode pH determinations is described. This apparatus, used in conjunction with a glass electrode cell, a rugged galvanometer, and a students' type potentiometer, makes it possible to determine the difference between the pH values of any pair of solutions, in the range from pH 1 to 9, with an accuracy of ± 0.005 pH.

Acknowledgment: The authors are indebted to Mr. Wasil Litvenko (Bioelectronic Laboratory, 16 Dow Street, New Haven, Connecticut) for his careful work in constructing the electronic bridge.

REFERENCES

1 Burr, H. S. and Mauro, A.: Millivoltmeters. Yale J. Biol., 1949, *21*, 249.
2 Dole, Malcolm: *The glass electrode.* New York, John Wiley & Sons, 1941, pp. 32-63.
3 Hitchcock, D. I. and Taylor, A. C.: The standardization of hydrogen ion determinations. J. Am. Chem. Soc., 1937, *59*, 1812.
4 Nielsen, C. E.: Measurement of small currents. Rev. Sc. Instrum., 1947, *18*, 18.

EXPERIMENTAL PRODUCTION OF CRETIN-LIKE RATS*

RUSSELL J. BARRNETT†

Introduction

Cretinism is a term frequently applied to forms of thyroid deficiency occurring *in utero* or early in extra-uterine existence. Experimental cretinism probably had its beginnings in the first recorded extirpation of the thyroid in young animals, which seems to have been performed by Raynard[22] in 1834. Two years later, Astley Cooper[6] published his observations on thyroid extirpation in pups. His animals recovered, but suffered from "stupidity and malaise." It was not until the careful work of Schiff that any extended observations were made. He demonstrated the first undoubted hypothyroid symptoms due to experimental thyroidectomy and attempted to improve the symptoms by thyroid grafts.[24] Following this work, observations of the effect of thyroidectomy on young animals were reported by many capable workers.[5, 16, 26] A difference in operative results has been shown between young and old animals, carnivora and herbivora.[13] More recently the results of thyroidectomy in newborn rats have been reported.[20, 25]

Other experimental methods have been used to produce cretins. Subtotal thyroidectomy of a pregnant animal was shown to result in a high degree of thyroid hyperplasia in the offspring.[11] If after subtotal thyroidectomy in pregnant dogs iodine was withheld from the diet, litters were produced which all showed extreme hyperplasia of the pups' thyroids and existence of cretin-like characteristics in the pups. If iodine was fed to the pregnant animals, the resulting litter was perfectly normal.[19] Harington[12] believes that the aforementioned method is precisely the way human cretins get their start, with the thyroid terminating in fibrous atrophy.

Morgan and Ivy[21] made use of an antithyroglobulin antibody in newborn rabbits and found that growth was retarded, and that the animals showed "shaggy hair, pot bellies and apathy." Thyroid glands in these animals were smaller than normal, but the histology was not reported. Administration of thyroxine to pregnant dogs and rats was said by Cunningham[7] to

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* From the Laboratory of Physiology. The data presented here are taken, in part, from a thesis submitted to the faculty of the Yale University School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine, 1948.

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produce cretin-like characteristics in the offspring; but no anatomical studies were done.

In 1941 MacKenzie, MacKenzie, and McCollum[15] found that the sulfonamides were goitrogenic despite a high intake of iodine. Astwood,[8] soon after, reported that thiourea derivatives, aminobenzines, and sulfonamides possessed similar goitrogenic properties. Treatment of experimental animals with these substances produced hyperplasia of the thyroid, a fall in basal metabolic rate, and histological changes in the pituitary closely resembling those which follow thyroidectomy. Thiouracil appeared to be the most potent thyroid depressant of these new drugs.[8]

Shortly after the discovery of the antithyroid drugs which produced goiter and hypothyroidism in experimental animals, it occurred to various investigators that these substances could be used to produce a cretin-like state. The first such experiments were reported by Hughes in 1944, who produced cretinism by injecting thiouracil subcutaneously into newborn rats.[14] Growth was inhibited markedly in these animals.

The following experiments were designed in an effort to elucidate further some changes in the physiology of the experimental cretin-like animal. They were set up as an alternative to thyroidectomy as a method of inducing hypothyroidism in a young animal. Since it has been established that thyroid activity can be inhibited by the use of antithyroid drugs, it was decided to use one of these drugs, thiouracil, to determine its effect upon the early development of the rat.

Methods

Female rats of the Sprague-Dawley strain were raised in a constant temperature room at 76°-78°F. When these rats weighed 180-200 gms., they were mated according to the method described by Long and Evans.[17] Vaginal smears were taken daily. If in estrus, these rats were immediately mated with virile males of the same strain. If sperm or cervical plug was found on the next vaginal smear taken within twelve hours, the female rats were assumed pregnant, and started on thiouracil. This drug was administered thoroughly mixed in the animals' diet (ground purina chow or calf meal), from which the treated animals were allowed to feed ad libitum. Three dosage levels of thiouracil were administered in this manner to three groups of pregnant female rats throughout the period of gestation and suckling, as follows: 0.25% (0.25 gm. thiouracil per 100 gm. of feed), 0.50%, and 1%. Although there were no significant differences in the number of progeny produced by the thiouracil-treated and normal female rats, all litters were reduced to six animals following birth. In the treated group of rats, at weaning, the same amount of thiouracil was fed to each of the progeny as was fed to the maternal rat in the diet as described above. Control litters of untreated animals were raised concurrently.

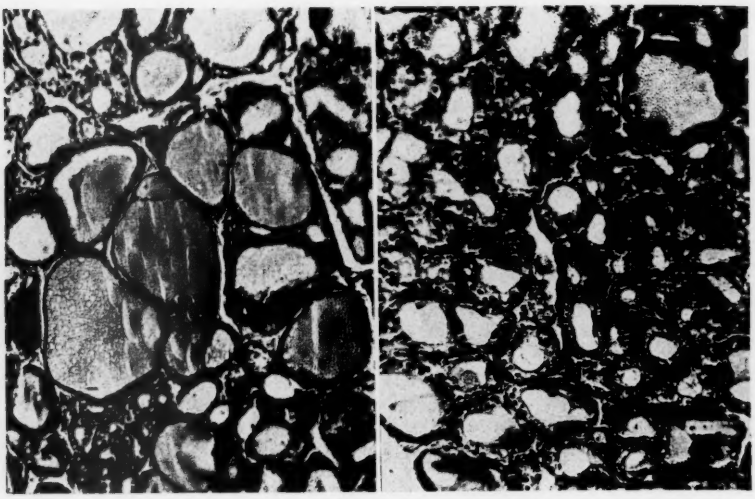

FIG. 1. Thyroid glands of normal and thiouracil fetus-fed adult rats.

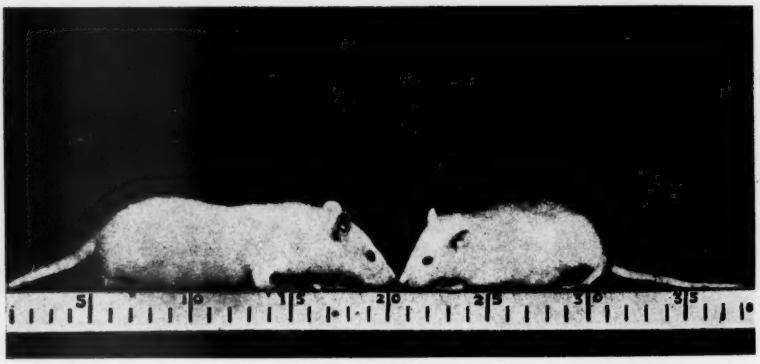

FIG. 4. Comparison of 35-day-old normal rat and cretin (0.25% thiouracil).

Results

Placental transmission. A group of eight normal pregnant rats was fed 0.5 gm. of thiouracil per 100 gm. of diet (0.5%) for twenty days. This amounted to an average of 1.485 gm. of thiouracil for this period per rat, or 0.074 gm. per rat per day. At the end of twenty days the pregnant rats were sacrificed and the fetuses collected, washed, and stored in an icebox. The same procedure was carried out on eight control pregnant rats and their litters, to which no drug was administered. All of these fetuses were then fed to four normal female adult rats of the same age and weight, at the rate of three fetuses each day per rat. All other food was withheld. At the end of six days these animals were sacrificed and the thyroid glands of the normal fetus-fed and thiouracil fetus-fed group were compared. The thyroids of the group fed on thiouracil fetuses showed hyperplasia, typical of that seen with thiouracil administration. This was not marked, but very definite nonetheless, and comparable with that seen in thyroids of animals fed about 0.1% thiouracil for a similar length of time (see Fig. 1). The thyroids of the rats fed normal fetuses did not vary histologically from the thyroids of untreated stock animals of the same age and sex.

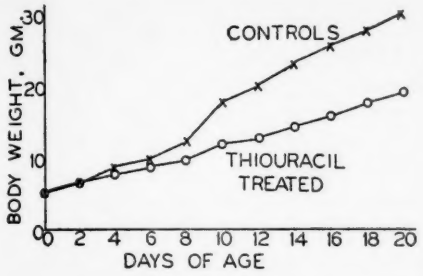

FIG. 2. Growth rates of treated and untreated suckling rats.

Transmammary effect. Six untreated mother rats of newborn normal litters were fed 0.5 gm. of thiouracil per 100 gm. of diet on the day of delivery of the normal litters and on succeeding days. This amounted to an average of 0.088 gm. of thiouracil per rat per day over a twenty-day period. The growth rate of these suckled animals was compared with the growth rate of six normal litters (see Fig. 2). By the tenth day there was an apparent difference in the daily weight curves; this difference became more marked by the time of weaning. Thyroid glands of the infant rats which were suckled by thiouracil-fed mothers were examined on the seventh and fourteenth days. Evidence of thyroid hyperplasia was found on the seventh day. The acinar cells were enlarged, the colloid was less abundant and stained less densely than that of the glands of the normal animals of

the same age. By the fourteenth day there was marked hyperplasia accompanied by enlargement and engorgement of the thyroid gland. At this time the weight of the thyroid glands of twenty treated animals averaged 5 mgm. greater than the average weight of the thyroids of the twenty untreated animals, which at this age was 1 to 1.5 mgm.

Effect on general development: Group A. Thiouracil at the dosage level of 0.25 gm. per 100 gm. of diet was fed to twelve female rats as soon as pregnancy was established. This treatment was continued following parturition until weaning, at which time the same dose was administered, mixed in the feed, to the young. The total dosage amounted to an average of 0.035 gm. of thiouracil per day per adult rat through the periods of pregnancy and lactation. The young hypothyroid animals ingested an average of 0.021 gm. of thiouracil per day following weaning. At birth the treated newborn rats showed no significant difference in weight from those of the control animals. The average birth weight was 0.5 gm. less than that of the control animals, but was within the range of normal variation. During the first few days there was no remarkable difference in growth rate. After the third or fourth day, however, it was apparent that growth, as measured by body weight, was inhibited in the treated animals. By the fourteenth day the difference was very apparent, and marked by the twentieth day. After weaning, the growth rate decreased still more; but in the ninety days under observation, the growth of these rats had not stopped (Fig. 3). During the forty days recorded, although significantly behind the normal animals in growth and weight gain, these animals did not lag behind the controls in the appearance of hair, opening of eyes, and date of weaning. It was noted, however, that the hair persisted as soft fur instead of changing to adult coat, usually found in the third week of life. The shape of the head remained blunt (see Fig. 4). Neurological manifestations were noted, in that the gait of these animals was ataxic at varying times after the thirty-fifth day. The body temperature was lower than that of the control animals, and the skin

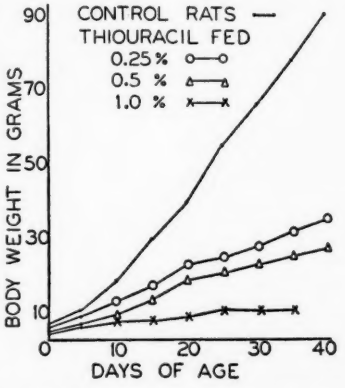

FIG. 3. Growth rate of normal animals and those fed varying concentrations of thiouracil.

had a peculiar thickness and loss of elasticity. The animals would huddle together when sleeping. They were aroused slowly by a tactile stimulus but, once aroused, showed a brief, unexpected burst of activity. In total activity they were obviously retarded in comparison to the normal animals, a retardation which at times approached lethargy. There was no mortality in this group, except for a small number succumbing to maternal cannibalism.

Group B. Twelve pregnant female rats were fed 0.5% thiouracil as described previously. This was an average of 0.071 gm. of thiouracil per day per rat for the adult, throughout pregnancy and lactation. After weaning, the cretin-like progeny ingested an average of 0.037 gm. of thiouracil per rat per day. The newborn rats of this group averaged 1 to 1.5 gm. less in body weight than the newborn controls. Developmental rate was even more profoundly depressed in this group than in those treated with 0.25% thiouracil (see Fig. 3). Typical cretinoid appearance was noted earlier and was more marked. Uncoördinated stiff gait and muscle movements appeared earlier and were more marked than in the previous group. Soft, immature fur persisted throughout the life span of these animals. The weaning date was postponed because of retarded development until about the twenty-fifth day of life. The growth plateau was reached at varying times in these animals, from the fifty-second to the seventy-ninth day, at which time they remained at a steady weight or lost weight. In general, these animals showed the same manifestations of hypothyroidism as the previous group, except that here they were more marked, more severe, and appeared earlier. The mortality in this group of animals between the times of weaning and reaching the growth plateau was zero. Death was an inevitable sequel from one to three weeks after growth had ceased, even if thiouracil therapy was withdrawn. There was approximately a fifty per cent mortality among the newborn because of maternal cannibalism. Withdrawal of thiouracil therapy was carried out in two groups of these animals whose growth had not stopped at the twentieth and fortieth day of therapy. Thereupon growth rate was very rapidly accelerated, with eventual loss of cretinoid characteristics.

The thyroid glands of newborn treated animals appeared slightly enlarged and grossly hyperemic. Microscopically, two types of changes were found, i.e., hyperplasia of cells, and absence of colloid in comparison with thyroids of normal newborns. The thyroids of the normal animals consisted of solid cell masses with little connective tissue in some cases, while others suggested beginning differentiation into solid cell cords, and

still others showed beginning development of colloid substance with follicles of varying number and size. The animals treated with thiouracil, on the other hand, showed absent or scanty colloid which stained lightly. The acinar cell height was increased above that seen in the normal; and the follicle formation was generally more regular and prominent, but disorderly hyperplasia often obscured the normal detail. Enlarged, hyperplastic, and colloidless goiters were found in all animals of advanced age. By forty days the thyroids of the thiouracil-treated animals were at least fifteen times the weight of the thyroids of normal animals. Hematoxylin-eosin stain was used in the thyroid preparations.

Group C. Fifteen pregnant female rats were fed 1% thiouracil as described. At this dose level these animals ingested 0.091 gm. of thiouracil per rat per day. In this group, six of the rats did not deliver, but resorbed their young. The nine remaining rats had prolonged gestation periods from twenty-three to twenty-seven days. Newborn mortality from maternal cannibalism was very high. Out of nine litters, only fourteen animals reached ten days of age; five of these animals lived to twenty-one days, and two of these lived to thirty-five days. Growth was almost at a standstill, the growth plateau was reached by the tenth day (see Fig. 3). Fur never developed completely, and the animals showed scattered areas of nude skin. Eyes opened seven to nine days late. No teeth developed. The animals could not walk, but crawled about the cages in newborn fashion. No animals in this series were weaned, and all succumbed to maternal cannibalism.

General effects. The food intake of the 0.5% thiouracil-fed adult rats was about 40% lower than that of the normal controls. This was also true of the thiouracil-fed pregnant rats and weaned cretins. Although this matter will be dealt with in another paper,[4] mention of it here seems warranted because a decreased food intake during gestation might well have a deleterious effect upon growth of the fetus; and following delivery, this decreased nutritional status might well have caused a decreased milk production in the maternal rat. Preliminary microscopic examination of the breasts of lactating thiouracil-fed rats who ate their young tends to support the idea of a poor state of function. No studies were performed on possible alterations of vitamin metabolism in the hypothyroid animals. No pathological changes were noted in the bones of the cretin-like rats in radiological studies. Thyroidectomy was not performed on newborn animals for comparison, but the results presented here compared well with those of Scow and Simpson.[25]

Discussion

Following the initial report of Hughes[14] on the production of an experimental condition similar to cretinism by the use of thiouracil, there have been a number of similar observations,[1, 9, 15] although propylthiouracil was said not to affect growth in beagle puppies.[20] Three points have been stressed generally; inhibition of thyroid function, production of goiter, and decreased growth rate. It appears obvious from the data already published and the results presented in this paper, that thiouracil depresses thyroid function in newborn rats so as to produce a cretin-like result. The method used here differs from previous reports in that the cretin-like rat was subjected to an athyroid developmental period, rather than having the function of the gland suppressed following birth. The use of thiouracil seems advantageous over thyroidectomy in that operative trauma and its sequella are obliterated; there is no question of parathyroid damage or thyroid remnants; and it presents the unique advantage of enabling one to thyroidectomize chemically both mother and litter before parturition occurs. There appear to be certain disadvantages in the method, however, among which a toxic effect of the drug is the foremost.

From the results mentioned, the response to thiouracil varies from animal to animal. This is best seen in the slight difference in growth rates of animals on the same dosage of drug, the time of plateauing of growth curves in the 0.5% thiouracil-fed animals (which varied from fifty-two to seventy-nine days), and the onset of neurological symptoms. There is no obvious explanation for this variation. One may postulate that these disturbances occurred because of accumulative toxic effects of the drug, or because of the inability of the experimental animals to carry on without their thyroid mechanisms, or possibly because of the loss of other organ functions dependent upon thyroid function. Concerning the toxicity of thiouracil, the animals on 0.5% thiouracil ingested an amount which approximates the dose which has been used for human subjects. Despite this fact, Williams *et al.*[27] have reported that the changes produced in animals by this dose, excepting agranulocytosis, can be attributed to decrease in thyroid function alone. No pathological changes were found in the liver, kidneys, lungs, or intestines of the autopsied animals.

Suggestive evidence is presented that thiouracil, in acting upon the thyroid, does not do so in all-or-none fashion, but does so quantitatively in relation to the dosage level used. This can be seen from the differences in the birth weights of the experimental cretins and the rates of development which varied with different doses. Among the animals given the largest

dose of thiouracil, it is clear that an adequate dose completely inhibited thyroid function as expressed in terms of the growth of a young animal. The reverse is also apparent: for when the treated animal is taken off therapy, growth very quickly spurts ahead. The difference in growth rate between the 0.25% and 0.5% thiouracil-fed animals may well be a difference in degree of suppression of thyroid function. This same fact may explain the irregularities seen in individual animals, and the fact that the growth plateau and onset of neurological symptoms varied in time. If, for example, because of inadequate absorption, or unequal distribution of concentration of thiouracil in the body tissues and fluids, or a chance intake of a smaller amount, the effective inhibiting concentration of thiouracil in the body tissues and fluids was lowered for a time, then the thyroid could manufacture a small amount of hormone which might produce the observed difference. It is not unreasonable, also, to assume that small amounts of thyroid hormone could be formed and liberated by the thyroids of the animals fed 0.25% thiouracil. This would be less in degree in the 0.5% thiouracil-fed animals, and virtually absent in animals fed the 1% dose. Differences in growth rates may then be related to the amount of thyroid hormone manufactured and liberated in the three different groups of animals.

The apparent changes in the thyroid glands of the newborn treated rats are presented as a further proof of placental transmission of thiouracil, and the beginning of development of hypothyroidism *in utero*. Because thyroid function begins in the fetal rat about the eighteenth day of gestation,[10] and because placental transmission of thiouracil had taken place at that time, there would be at least four days for thiouracil to act upon the fetal thyroid (provided birth of the fetus had not been delayed). This would further necessitate the postulation of the ability of the fetal hypophysis to produce thyrotropic hormone.[8] Reduced maternal thyroxine circulating to the fetus might cause hyperplasia and outpouring of thyrotropin of the fetal hypophysis. Lack of this hormone (i.e., thyroxine) to the fetus may also affect early development. Another possibility exists that fetal thyroid hyperplasia may be brought about by transmission of maternal thyrotropic hormone crossing the placental barrier. This possibility needs further confirmation, but it does not seem likely because the pituitary hormones are protein in nature and of large molecular size.

Summary

1. Experimental cretinism was produced by feeding thiouracil to pregnant female rats.

2. Thiouracil crossed the placental and mammary barriers.

3. Growth and early development of the offspring were retarded.

4. The thyroid glands of the newborn treated animals showed the hyperplastic effect of thiouracil.

5. The effect of thiouracil on the thyroid gland was a quantitative one; the degree of thyroid inhibition was directly related to the dosage of drug used.

References

1 Andrews, F. N. and Schnetzler, E. E.: Effect of feeding thiouracil to hens upon the thyroid gland of chicks. Endocrinology, 1945, *37*, 382.

2 Astwood, E. B.: Chemical nature of compounds which inhibit function of the thyroid gland. J. Pharm. Exp. Ther., 1943, *78*, 79.

3 Astwood, E. B., Sullivan, J., Bissell, A., and Tyclowitz, R.: Action of certain sulfonamides and of thiourea on function of thyroid gland of rats. Endocrinology, 1943, *32*, 210.

4 Barrnett, R. J.: Some aspects of the physiology of experimental cretin-like animals. (In preparation.)

5 Cameron, A. T.: *Recent advances in endocrinology*. Philadelphia, Blakiston Co., 1945, pp. 6-55.

6 Cooper, Astley: Guy's Hosp. Rep., Lond., 1836, *1*, 452.

7 Cunningham, B.: Experimental production of cretinoid progeny by hyperthyroidization of pregnant rats. J. Elisha Mitchell Sc. Soc., 1941, *57*, 85.

8 Fugo, N. W.: Effects of hypophysectomy in the chick embryo. J. Exp. Zool., 1940, *85*, 271.

9 Goldsmith, E. D., Gordon, A. S., and Charipper, H. A.: An analysis of the effects of continued thiourea treatment in pregnancy and the development of the offspring in the rat. Amer. J. Obst., 1945, *49*, 197.

10 Gorbman, A. and Evans, H. M.: The time of beginning function in the thyroid gland of fetal rats. Endocrinology, 1943, *32*, 113.

11 Halsted, W. S.: An experimental study of the thyroid glands of dogs with especial consideration of hypertrophy of the gland. Johns Hopkins Hosp. Rep., 1896, *1*, 373.

12 Harington, C. R.: *The thyroid gland*. London, Oxford University Press, 1933. xiii + 222 pp.

13 Hofmeister, F.: Untersuchungen über die Folgen des Schilddruseinverlustes. Beitr. klin. Chir., 1894, *11*, 441.

14 Hughes, A. A.: Cretinism in rats induced by thiouracil. Endocrinology, 1944, *34*, 67.

15 Hughes, A. A. and Astwood, E. B.: Inhibition of metamorphosis in tadpoles by thiouracil. Endocrinology, 1944, *34*, 138.

16 Liddell, H. S.: Effect of thyroidectomy on the intelligence of sheep. Proc. Soc. exp. Biol., N. Y., 1922, *19*, 343.

17 Long, J. A. and Evans, H. M.: The oestrous cycle in the rat and its associated phenomena. Memoirs of Univ. California, Vol. 6, 1922.

18 MacKenzie, J. B., MacKenzie, C. G., and McCollum, E. V.: Effect of sulfanylguanidine on the thyroid of the rat. Science, 1941, *94*, 518.

19 Marine, D. and Lenhart, C. H.: Effect of the administration or withholding of iodine-containing compounds in normal, colloid, or actively hyperplastic thyroids of dogs. Arch. Int. M., 1909, *4*, 253.

20 Meyer, E.: Inhibition of thyroid function in beagle puppies by propylthiouracil without disturbance of growth or health. Endocrinology, 1947, *40,* 165.

21 Morgan, J. E. and Ivy, A. C.: Production of cretinism by thyrocytotoxins. Proc. Soc. exp. Biol., N. Y., 1934, *31,* 1139.

22 Raynard: *Comptes Rendus des Traveux de l'Ecole Royal Vétérinaire de Lyon pendant l'année scholaire,* 1834.

23 Salmon, T. N.: Effect of pituitary growth substance on the development of rats thyroidectomized at birth. Endocrinology, 1941, *29,* 291.

24 Schiff, M.: Résumé d'une série d'expériences sur les effets d'ablation des corps thyroïdes. Rev. méd. Suisse rom., 1884, *4,* 2.

25 Scow, R. O. and Simpson, M. E.: Thyroidectomy in the newborn rat. Anat. Rec., 1945, *91,* 209.

26 Tatum, A. L.: Morphological studies in experimental cretinism. J. Exp. M., 1913, *17,* 636.

27 Williams, R. H., Wienglass, A. R., Bissell, G. W., and Peters, J. B.: Anatomical effects of thiouracil. Endocrinology, 1944, *34,* 317.

DESCRIPTION OF TOXIN IN TOXOPLASMOSIS*

DAVID WEINMAN AND H. J. KLATCHKO

Toxin formation is such a common characteristic of bacteria as to make it seem plausible that other groups of microörganisms also produce toxins. Yet in the protozoa, for example, it has not been possible to demonstrate any producer of potent toxin—this despite energetic search in malaria and other diseases where various data have strongly suggested the action of a toxin. Failure to demonstrate toxin may occur because there is no toxin to demonstrate, and this seems to be the view generally assumed. The alternative is that the methods have not been suitable.†

It is the purpose of this paper to describe briefly and in a preliminary way a toxin which develops *in vivo* in animals infected with *Toxoplasma*. Whether this is to be interpreted as evidence for or against the protozoan nature of *Toxoplasma* will be discussed elsewhere. For convenience at present, the toxin will be referred to as "Toxotoxin" and considered a single substance, it being well understood that the toxin may later prove to have several components.

The toxoplasma strain utilized is the RH strain of human origin; it was originally isolated by A. B. Sabin about eight years ago[2] and was kindly furnished to the authors by H. A. Feldman. At present it is highly pathogenic for mice and rabbits, whereas lethal doses for these animals produce in rats the chronic type of latent infection with pseudocysts in the brain which has previously been described.[4]

Infection was maintained by serial mouse passage. The animals were injected intra-abdominally, and the peritoneal fluid which developed subsequently served for passage material. The customary inoculum was 0.1 cc. of a 10% dilution in saline of the supernatant obtained after centrifuging for thirty minutes at 3600 r.p.m. So treated, mice usually survived about five days, showing a copious ascites at autopsy.

Peritoneal fluid is usually scanty until about forty-eight hours before death; then it increases quite rapidly and, if the mice are held until mori-

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† *Sarcocystis* produces a potent toxin, and an exception to the preceding statement may have to be made for this microörganism. *Sarcocystis*, however, is not universally held to be a protozoön.[3]

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bund, 3 cc., or at times more, may be harvested from each mouse. The physical characteristics of the fluid vary considerably; some samples are viscid and coagulate spontaneously, either immediately or on standing; others are watery and show little coagulum on boiling.

The toxin is contained in the peritoneal fluid and can most readily be put in evidence by intravenous injection in mice. The manifestation is unmistakable; death invariably results when adequate doses, ranging from 0.1 to 0.5 cc., of whole peritoneal fluid supernate are so injected.

Spectacular rapidity of action is an outstanding characteristic of the toxin. When a lethal dose is injected intravenously, the animal is usually dead before it can be removed from the holding apparatus—sometimes before the needle is out of the vein. This occurs with minimum lethal doses established to the nearest 0.1 cc.; with a decrement of 0.1 cc. in the inoculum the animal usually shows no immediate ill effect. Occasional samples act more slowly and require ten to fifteen minutes to cause death. In general, if an animal survives for thirty minutes, it does not subsequently die from the acute effects of the toxin.

Symptoms are hard to follow in the most acute forms; the animal falls to one side, makes a few spasmodic movements of fore and hind quarters, and is dead. When the action is slower, the animal drops where deposited; if on the ventral surface, it will be prone and not raised upon its legs. It is inert, does not move, although it may respond to stimulation, and just before death falls to one side and has the kicking movements described. Respiratory distress is often a conspicuous symptom in animals which do not die. In such, breathing may be periodic with six to ten slow inspirations followed by a number of very hurried breaths, then the slow inspirations begin once more, and so on.

Peritoneal fluid harvested from different mice varies in toxicity. The usual lethal dose lies between 0.1 and 0.5 cc. for a mouse weighing 20 to 25 grams. In general, samples collected from moribund mice when the fluid is most copious contain less toxin in a unit volume. This direct relation between total volume and size of lethal dose suggests that the ascitic fluid collecting in the abdomen during the final forty-eight hours may have a diluting effect. A single mouse usually furnishes from four to ten lethal mouse doses by the method described.

The route of inoculation is of prime importance; the data thus far presented pertain to intravenous inoculations. Intraperitoneal injections of as many as five lethal intravenous doses are tolerated perfectly by mice. The range of susceptible normal animals is not yet ready for report. Toxoplasma-

infected mice tested one to two days before death require approximately the same dose of toxin for lethal effect as do normal controls, indicating neither increased susceptibility nor resistance during the acute phase of the disease.

Toxotoxin is produced *in vivo;* no answer is available concerning its production *in vitro* since the organism has not been grown on cell-free medium. Whether it is an endotoxin, an exotoxin, or some type of reaction product between microörganism and host, cannot be answered as yet. There is no positive evidence suggesting an endotoxin. In numerous experiments the injection of toxoplasmata, washed in saline and then lysed by freezing and thawing, has not given toxic symptoms. While doses in excess of 6×10^7 intact organisms have not been given, it should be noted that the centrifuged supernatant from which these organisms were removed was highly toxic. Furthermore, supernatant toxic fluid stored with organisms has not changed detectably in titer. These data suggest that if an endotoxin is present at all in the washed toxoplasmata, it is not highly concentrated and is probably not the toxotoxin which is the subject of this report. It is known that washed toxoplasmata contain antigenic material which elicits a skin reaction in infected persons and animals.[4] Thus far toxotoxin resembles the toxin(s) formed within the body of animals infected with anthrax rather than the more usual bacterial toxins.

Toxotoxin is relatively stable. It is not decreased detectably by storage at room temperature or for twenty days at 5° C. It resists heating to 56° C. for thirty minutes and immersion in a boiling water bath for a half hour. Activity is diminished, however, by autoclaving at 15 lbs. pressure (121° C.) for twenty minutes.

Toxicity is not appreciably reduced by eight minutes of exposure to bactericidal ultraviolet rays (2537 Å, approximate delivered intensity 346 microwatts per cm^2*). It has not been detectably altered by incubation with neutral formalin to a final concentration of 0.1% for four hours at 37° C., whereas after one month at 37° C. with formalin to 0.3% final concentration, the titer was reduced to about one-half. Sodium sulfadiazine is effective in the treatment of toxoplasmosis[5] but has shown no activity against the toxin. Mice first injected intra-abdominally with 10 mg. of drug, then, three hours later, with a minimum lethal dose of toxin previously incubated for thirty minutes at 37° C. with sodium sulfadiazine to 0.5% final concentration, died promptly.

The toxin may be frozen and dried in the frozen state. It is then stable,

* Calculated from manufacturer's data.

may be stored and, when reconstituted, is fully active. Such a method is one means of concentration of relatively dilute toxins; the rehydrated powders closely approximate the calculated lethal doses.

As yet not very much is known about the chemical constitution of toxotoxin. Activity is completely destroyed by the action of crystalline trypsin so that it is a protein, or at least contains a trypsin digestible protein fraction without which activity is nil. The toxin is stable at the pH of trypsin activity, so that loss of activity is not a pH effect. Dialyzed against saline in a collodion bag for ninety-four hours, the toxin did not diffuse out, for the lethal dose of bag contents increased only as the dilution factor. This indicates that toxicity is not associated with a substance of low molecular weight.

Studies on the antigenicity of the toxin have not yet been completed. It seems not impossible that toxotoxin plays some part in the lethal effects of the disease toxoplasmosis, bears upon the pathology of the disease, and is concerned in some of the immunological diagnostic tests in use. These subjects are now under investigation and will be the subject of subsequent reports.

Conclusions

1. A toxin is produced in animals infected with *Toxoplasma*. It has provisionally been named toxotoxin, cognizance being taken that it may prove to have more than one component.

2. The toxin develops *in vivo* and can be obtained several days before and up to death.

3. It is produced in the peritoneal fluid of infected animals and is lethal to mice by the intravenous route.

4. Notable characteristics of toxotoxin are its extreme rapidity of action and its heat stability.

5. All data thus far obtained suggest that it is not an endotoxin.

6. It is probably a protein or else contains a protein fraction without which activity is lost. Toxicity is not associated with a substance of low molecular weight.

References

1 Frenkel, J.: Dermal sensitivity to toxoplasma antigens (toxoplasmosis). Proc. Soc. Exp. Biol., N. Y., 1948, *68*, 634.
2 Sabin, A. B.: Toxoplasmic encephalitis in children. J. Am. M. Ass., 1941, *116*, 801.
3 Spindler, L. A. and Zimmerman, H. E., Jr: The biological status of *Sarcocystis*. J. Parasitol., 1945, *31*, 13 (Dec. Supplement).
4 Weinman, D.: Chronic toxoplasmosis. J. Inf. Dis., 1943, *73*, 85.
5 Weinman, D. and Berne, R.: Therapeutic cure of acute experimental toxoplasmosis in animals. J. Am. M. Ass., 1944, *124*, 6.

THE AGE INCIDENCE OF POLIOMYELITIS IN CONNECTICUT 1921-1947*

JULIAN IRVING PICHEL

During the past twenty years there has been occasional discussion in medical literature about the changing age incidence of poliomyelitis. In general this discussion has dealt with the evidence that poliomyelitis is no longer a disease which is confined more or less to infants. Comments on this have come from Australia,[2] Denmark,[8] Germany,[24] Sweden,[1] and from this country.[4, 6, 7, 11, 16, 20, 22]

In reviewing this subject briefly, one finds that at the turn of the century, "infantile paralysis," as it was then called, was considered to be "rather rare after the age of six."[19] Indeed more than 50 per cent of the cases were under two years of age. In one of the earliest epidemics in the United States, occurring in Vermont in 1894, 90 out of the 120 cases whose ages were given were under the age of six.[3] Today, however, in Scandinavia[1, 8] as well as in northern United States,[7] poliomyelitis is seemingly no longer a disease of infants; perhaps 70 to 80 per cent of the total cases occur in people over the age of five, and as high as 25 per cent in people over the age of 15 years.

This gradual shift in the age of the patients has apparently not taken place in some parts of the world, but in others it has progressed at varying rates during the past thirty years or so. The State of Massachusetts furnishes data in this regard, as originally exemplified by the studies of Forsbeck and Luther,[6] who observed no apparent change in age distribution of cases until 1918, twenty-five years after the initial epidemic in that state. After this time there occurred a marked decrease in the relative number of cases in the age group under five years, with a "compensatory" increase in the relative number of cases in the 5-14 age group. The percentage of cases in the older age groups was found to remain more or less constant.

Subsequently, in Connecticut, Knowlton[11] reported an increased percentage of cases over five years of age occurring in the 1931 series of cases as compared with the percentage distribution of cases in this age group for earlier years. And Wenner,[22] in a study of poliomyelitis in the city of New Haven and adjacent areas, found that about 25 per cent fewer children under five years were reported to have had the disease in 1943 as compared with 1916. A reciprocal increase was seen in the 5-9 and 10-15 age groups.

But it is not enough to indicate that the percentage of cases of poliomyelitis in the different age groups has shifted over the years—because, unless their shift is analyzed on the basis of *age-specific rates,* it may be found to be more apparent than real. In

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this connection Horton and Rubenstein[10] have again reviewed the Massachusetts data and have likewise found a decrease in the incidence of poliomyelitis there among children under five years of age, most marked between 1920 and 1930. They concluded, however, that the apparent variation in age distribution in Massachusetts has been due almost entirely to changes in the age distribution of the population and to an increase in the reporting of non-paralytic cases, and that there has been no essential change in the epidemiological behavior of the disease in that state.

Dauer[4] studied the same problem in a series of cases from five northern states (including Massachusetts and Connecticut) and five southern states, and found a consistent decrease in the proportion of cases in the age group under five years in each of the northern states since about 1916. In his analysis of *age-specific rates,* however, he found little evidence of a shift, i.e., a consistent rise or fall in any of the age groups except for the group under five in Massachusetts. He found changes in the age distribution of fatal cases similar to those in total number of cases except for a greater proportionate decrease in the deaths among the 0-4 age group. Consequently it would appear that the age specific mortality rates from Dauer's study do not indicate a consistent change since 1920 *except for those under five,* where a considerable reduction in mortality rate has occurred. This finding led Dauer to suggest that perhaps the disease is actually, as well as proportionally, becoming less frequent in this youngest age group.

Gilliam[7] also has recently examined the changes in *fatal* poliomyelitis occurring in the Death Registration States of 1910, during the period 1910-1940. His findings agree with those of Dauer, but he concludes that the decline in mortality in those under five years of age is a change that does not necessarily apply to the non-fatal disease.

Before one can account for these relative changes in the age incidence of poliomyelitis, a number of variables should be recognized. Thus the age incidence of poliomyelitis also varies with the density of population. Lavinder, Freeman, and Frost[12] and Nicoll,[14] in their reviews of the 1916 epidemic in New York city and state, confirmed previous observations on the differences in age distribution of cases between rural and urban communities, namely, that a greater proportion of cases occurred in older age groups in *rural* areas, with a higher incidence of the disease among young children in *urban* areas. Forsbeck and Luther[6] noted that over a seventeen-year period in Massachusetts the median age in the most sparsely populated density group was about eight, while the median age was about four in the most densely populated areas. But it is questionable whether the differences between rural and urban populations are as definite in this country today as they were a generation ago. Horton and Rubenstein[10] observed that as far as the age of poliomyelitis patients in Massachusetts is concerned, such differences are rapidly disappearing. This may not apply universally, however, for, in a single epidemic in western New York state and Pennsylvania in 1944, Paul[15] analyzed the age specific rates for urban and rural populations and found the high attack rates in the urban infants (0-4 group) still demonstrable in appreciable degree.

Granted that it is difficult to generalize about poliomyelitis from any single epidemic or series of cases from any single area, it is the aim of this study to determine whether or not an analysis of Connecticut data covering a period of some twenty-five years would lead to conclusions the same as

those of Horton and Rubenstein.[10] To this end an attempt has been made to measure the following items:

(i) The epidemic history of poliomyelitis in Connecticut;
(ii) The age distribution of cases each year since data became available;
(iii) The age distribution of fatal cases each year since data became available;
(iv) Population shifts between age groups and the effect of this upon the distribution of cases and fatalities;
(v) Age trends in rural as opposed to urban populations.

Methods

In order that data might be secured for this study, pertinent records of the Connecticut State Department of Health were made available through the kindness of the late Dr. E. E. Lamoureux, Director of the Bureau of Preventable Diseases, and his staff.

Case records. The material reviewed included morbidity and mortality reports of all cases of poliomyelitis which were reported to the Department of Health subsequent to 1900. Early records (1900-1920) are scanty, in fact poliomyelitis did not become a reportable disease in Connecticut until 1916. Not until 1920 did the reports become adequate for analysis. Consequently the study was limited to the period from 1921 to 1947. The age of the patient and time and place of onset of each individual case were obtained from these records. Unfortunately, adequate data concerning the differentiation of cases into paralytic and non-paralytic types were not available.

Population estimates of Connecticut towns used in the calculation of age-specific rural-urban analyses were obtained from reports published monthly in the Connecticut Health Bulletin by the Bureau of Vital Statistics of the State Department of Health and from Federal Census Figures. Populations of age groups used in the calculation of age-specific morbidity and mortality rates were from arithmetic estimates, based on Decennial Federal Census Figures, prepared by Eleanor J. Macdonald, Research Statistician of the Connecticut State Department of Health.

In attempting to correct the percentage distribution of cases for the population changes which have occurred within the various age groups during the period of time studied, mean annual, age-specific rates per 100,000 of the population were obtained for five-year periods. From the age-specific rates, the theoretical number of cases which could be expected in a normally distributed population was derived by adjusting these rates to a standard population, which, for this purpose, can be considered to be the population of age groups similar to those of the 1900 census in Connecticut. In comparing rates adjusted in this manner, any differences between one age group and another, or between the same age group in different years, may be considered to be independent of the effects of population changes.[21] Mortality rates were calculated in the same way as were case rates.

Urban and rural cases. In order to obtain a general picture of differences in rural and urban distribution, cases were divided into three groups—those from cities of

100,000 or more people, those from urban and town areas of 10,000 to 100,000 people, and the remainder from towns and rural areas of less than 10,000 people. Age-specific

TABLE 1

POLIOMYELITIS IN CONNECTICUT 1916-1947

Year	*Cases*	*Deaths*	*Case rate*	*Mortality rate*	*Case fatality rate*
1916	951	277	73.6	21.4	29.1
1917	29	16	2.1	1.2	55.2
1918	51	16	3.6	1.1	31.4
1919	12	10	0.8	0.7	83.3
1920	41	23	2.9	1.7	56.1
1921	86	25	6.1	1.8	29.1
1922	47	16	3.2	1.1	34.0
1923	88	10	6.0	0.7	11.4
1924	120	23	8.0	1.5	19.2
1925	50	20	3.3	1.3	40.0
1926	32	6	2.1	0.4	18.8
1927	188	16	11.9	1.0	8.5
1928	70	12	4.8	0.7	17.1
1929	18	8	1.1	0.5	44.4
1930	70	17	4.3	1.1	24.3
1931	1,136	90	69.3	5.5	7.9
1932	29	7	1.8	0.4	24.1
1933	78	3	4.6	0.2	3.8
1934	14	2	0.8	0.1	14.3
1935	402	24	23.3	1.4	5.7
1936	16	4	0.9	0.2	18.8
1937	101	8	5.9	0.5	7.7
1938	18	0	1.1	...	...
1939	28	2	1.5	0.1	7.1
1940	16	3	0.9	0.2	12.5
1941	114	2	6.6	0.1	1.8
1942	46	2	2.7	0.1	4.3
1943	378	22	21.7	1.2	5.7
1944	219	10	12.5	0.5	4.6
1945	214	9	12.2	0.5	4.2
1946	212	16	6.8	0.9	13.2
1947	133	7	7.5	0.4	5.3

rates for cases from the earliest five-year period of the study and for cases from the last five-year period of the study were calculated from each of the three areas.

Diagnostic nomenclature. There has been a decided shift in clinical concepts during the past twenty-five years as to what constitutes a case of poliomyelitis. For instance,

more cases of non-paralytic poliomyelitis are so designated in the 1930's and 1940's than was the situation in the 1920's. But, on the other hand, it has become increasingly difficult to define a case of non-paralytic poliomyelitis. For instance, in the present day when extensive muscle testing is performed, transient weaknesses lasting only a day or two have been designated as "paralytic cases" and thus the problem of definition has again shifted with a tendency for an increase in the relative percentage of paralytic cases. No correction for this variability of diagnostic nomenclature has been applied in this study.

Results

The first recorded epidemic of poliomyelitis in Connecticut was in 1905; two subsequent epidemics of major proportions have occurred since that time, in 1916 and 1931. The years between these epidemics have been characterized by intermediate peaks every two or three years. The incidence of the disease throughout its reportable history has been variable, with the total yearly number of cases ranging from twelve in 1919, to 1,136 in the epidemic of 1931. Table 1 lists the yearly number of cases and deaths with corresponding crude rates per 100,000 since 1916.

It is apparent here that the annual number of deaths has decreased somewhat and case fatality rates have shown a definite decline. This latter is probably due to gross under-reporting of clinical cases during the period immediately after 1916. Such an explanation is reasonable when one encounters a case fatality rate of some 40 per cent or more (!) during an epidemic year. It could either indicate that a strain of poliomyelitis virus of unprecedented killing power was about, or that only the more severe cases were being reported as examples of the disease. Of the two explanations the latter seems the more likely.

Chronological trends in the percentage of cases in various age groups. Table 2 shows the number of cases by age groups and their percentage distribution. The latter have been calculated from the number of cases with ages given, rather than from the entire total. There was a rapid decline in the percentage of cases in the 0-4 age group from 67 per cent of the total in 1921 to 15 per cent in 1947. This trend was most marked until about 1935, after which time the decline has been less obvious. A compensatory increase in the percentage of cases in the 5-9 and 10-14 age groups has occurred, with little consistent change in the older (greater than 15) age groups. The proportion of cases in the 5-9 group rose from 10 per cent of the total in 1921, to 50 per cent in 1934, after which time there has been a suggestion of a decline similar to that noticed in earlier years for the 0-4 age group.

The general age distribution does not seem to have been altered in years of particularly high incidence. Most of the deviations from the general

TABLE 2

NUMBER AND PERCENTAGE OF CONNECTICUT CASES IN VARIOUS AGE GROUPS DURING THE PERIOD 1921-1947

	Number of cases by age group		*Age groups*										
			0-4		*5-9*		*10-14*		*15-19*		*20 plus*		
Year	*Age not given*	*Total with age given*	*no.*	*%*	*no.*	*%*	*no.*	*%*	*no.*	*%*	*no.*	*%*	*Total*
1921	20	66	44	66.7	7	10.6	4	6.0	4	6.0	7	10.6	100
1922	3	44	23	52.3	8	18.2	6	13.6	3	6.8	4	9.1	100
1923	3	85	51	60.0	23	27.0	9	10.6	1	1.2	1	1.2	100
1924	1	119	67	56.2	27	22.7	15	12.6	1	0.9	9	7.6	100
1925	0	50	17	34.0	20	40.0	6	12.0	2	4.0	5	10.0	100
1926	0	32	17	53.1	7	21.9	4	12.5	1	3.1	3	9.4	100
1927	18	170	70	41.2	57	33.5	22	12.9	5	3.0	16	9.4	100
1928	3	67	31	46.3	13	19.4	9	13.4	10	14.9	4	6.0	100
1929	0	18	5	27.8	5	27.8	4	22.2	0	0.0	4	22.2	100
1930	2	68	22	33.8	17	25.0	9	13.2	10	14.7	10	14.7	100
1931	7	1,129	414	36.6	412	36.5	178	15.8	69	6.1	56	5.0	100
1932	0	29	8	27.6	8	27.6	8	27.6	2	6.9	3	10.3	100
1933	0	78	13	16.7	36	46.2	14	17.9	7	9.0	8	10.2	100
1934	0	14	2	14.3	7	50.0	2	14.3	1	7.1	2	14.3	100
1935	16	386	118	30.5	137	35.5	70	18.2	35	9.1	26	6.8	100
1936	0	16	5	31.25	7	43.8	3	18.8	1	6.25	0	0.0	100
1937	2	99	28	28.3	36	36.4	22	22.2	5	5.0	8	8.1	100
1938	1	17	1	5.9	6	35.3	3	17.6	3	17.6	4	23.6	100
1939	0	28	6	21.4	10	35.7	6	21.4	2	7.2	4	14.3	100
1940	0	16	2	12.5	7	43.8	5	31.5	2	12.5	0	0.0	100
1941	2	112	23	20.5	32	28.6	29	26.0	17	15.2	11	9.7	100
1942	0	46	12	26.0	20	43.5	11	23.9	2	4.4	1	2.2	100
1943	6	372	100	26.9	112	28.6	100	26.9	32	8.7	28	7.3	100
1944	4	215	35	16.3	77	35.8	53	24.6	24	11.2	26	12.1	100
1945	7	207	29	14.0	59	28.5	53	25.6	27	13.0	39	18.9	100
1946	5	116	23	19.8	38	32.7	30	25.9	7	6.0	18	15.6	100
1947	5	128	20	15.7	41	32.0	29	22.7	19	14.8	19	14.8	100

trend are seen in years of especially low incidence, where there is a tendency for more cases to be distributed among the older age groups. Perhaps, as in the case of the bizarre fatality rates previously mentioned, much of this may be due to the under-reporting of clinical cases.

TABLE 3

MEAN ANNUAL AGE-SPECIFIC POLIOMYELITIS CASE RATES IN CONNECTICUT WITH ADJUSTED PERCENTAGE DISTRIBUTION FOR FIVE-YEAR PERIODS 1921-1945

Age group	*Number of cases*	*Population age group*	*Age-specific rate mean annual*	*Adjusted no. cases*	*% Dist.*
1921-1925		*1923*			
0-4	200	147,558	27.0	124.0	55.6
5-9	87	143,056	12.2	52.5	23.5
10-14	40	133,405	6.0	23.0	10.3
15-19	11	119,219	1.8	7.1	3.2
20+	26	905,269	0.6	16.5	7.4
1926-1930		*1928*			
0-4	145	137,093	21.2	96.0	45.0
5-9	99	149,820	13.3	57.0	26.7
10-14	48	152,431	6.3	24.4	11.4
15-19	26	139,864	3.7	14.5	6.7
20+	37	982,408	0.8	21.7	10.2
1931-1935		*1933*			
0-4	555	125,629	88.6	406.0	39.7
5-9	600	141,507	85.0	365.0	35.7
10-14	272	153,512	35.4	135.0	13.4
15-19	114	150,149	15.1	58.0	5.7
20+	105	1,066,145	2.0	57.0	5.5
1936-1940		*1938*			
0-4	42	113,514	7.4	34.0	29.4
5-9	66	123,138	10.8	46.0	39.8
10-14	39	142,621	5.4	21.0	18.2
15-19	13	153,523	1.7	6.5	5.6
20+	16	1,155,764	0.3	8.0	7.0
1941-1945		*1943*			
0-4	199	101,391	39.4	180.0	26.8
5-9	300	104,762	57.8	250.0	37.1
10-14	246	131,726	37.4	143.0	21.4
15-19	102	156,906	13.0	50.0	7.4
20+	105	1,245,434	1.7	49.0	7.3

Chronological trends in age-specific rates. Any interpretation of the figures presented in Table 2 must necessarily be withheld until the possible effects of changes in population distribution between the various age groups have been examined. Wenner[29] observed that in the city of New Haven, because of the lowered birth rate in recent years, there were about 30 per cent fewer children under the age of fifteen in 1943 than there were in 1920. If a similar reduction in the younger age groups has taken place in Connecticut as a whole, then the apparent decrease within these age groups may not be a real change at all. To correct for such population changes, age-specific rates and the adjusted number of cases were determined for five-year periods as described; they are presented in Table 3. Percentage distributions from this table are shown in Figure 1. When the percentage distribution of poliomyelitis cases is adjusted for population changes as in Table 3, the downward trend in the 0-4 age group becomes less marked than the decrease suggested by Table 2; yet a definite change remains. The 5-9 group shows the same initial rise and subsequent lesser decline, the 10-14 group shows a consistent rise, and the older age groups remain more or less constant.

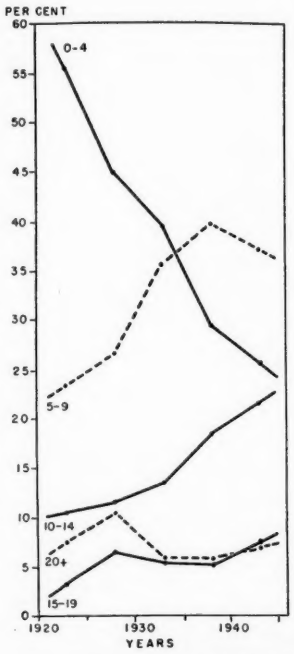

FIG. 1. Percentage distribution of age-specific case rates for poliomyelitis in Connecticut, 1921-1945. Each line designates the trend for a given age group: 0-4; 5-9; etc.

Age-specific mortality rates. If the age-specific case rates and the theoretical adjusted number of cases derived therefrom can be said to rule out the effects of population changes, then it can be said that the trends in poliomyelitis age distribution are not entirely dependent upon alterations in the age of the population. In other words, to explain these trends additional factor or factors must be operative. It is believed that the reporting of *non-paralytic* cases has increased during the period of time covered by this study, and that it is probable, but not proved, that a larger proportion of these cases fall into the older than the younger groups. It would thus be

important to measure the age trends in a series of exclusively paralytic cases. In the absence of these data, however, it would seem that the age-

TABLE 4

MEAN ANNUAL AGE-SPECIFIC POLIOMYELITIS MORTALITY RATES IN CONNECTICUT WITH ADJUSTED PERCENTAGE DISTRIBUTION FOR FIVE-YEAR PERIODS 1921-1945

Age group	*Number deaths*	*Population*	*Mean annual mortality rate*	*Adjusted no. deaths*	*% Dist.*
1921-1925		*1923*			
0-4	41	146,558	5.6	25.4	40.0
5-9	11	143,056	1.5	6.6	11.0
10-19	20	252,624	1.5	12.0	19.0
20+	28	905,269	0.7	19.0	30.0
1926-1930		*1928*			
0-4	21	137,093	3.1	14.0	52.0
5-9	5	149,820	0.7	2.9	10.8
10-19	13	292,295	0.9	2.9	10.8
20+	12	982,408	0.2	7.0	26.4
1931-1935		*1933*			
0-4	29	125,629	4.6	22.6	30.0
5-9	39	141,507	5.5	23.7	35.7
10-19	33	303,661	2.2	16.7	18.1
20+	23	1,066,145	0.4	12.5	5.5
1936-1940		*1938*			
0-4	3	113,514	0.6	2.7	22.7
5-9	7	123,138	1.1	4.9	41.1
10-19	4	296,144	0.3	2.1	17.7
20+	3	1,155,764	0.1	2.2	18.5
1941-1945		*1943*			
0-4	2	101,391	0.4	1.8	6.0
5-9	9	104,762	1.7	7.5	28.2
10-19	23	288,632	1.6	12.2	46.0
20+	11	1,245,434	0.2	5.1	19.8

specific mortality rates would give supplementary information of value, since these cases should not be influenced particularly by the effects of the increased reporting of non-paralytic poliomyelitis.

Table 4 gives the age-specific mortality rates of age groups from 1921-1945. Percentage distribution of the theoretical number of deaths derived from these rates is presented in Figure 2. The percentage distribution of mortalities reveals the same general trends as do the total cases, except for the initial increase in percentage distribution of the 0-4 age group, and the corresponding early decrease in percentage distribution of the 10-19 and 20 plus groups. The age-specific mortality rates have little consistent variation in any of the age groups with the exception of the 0-4 group, which shows a general decline since 1921.

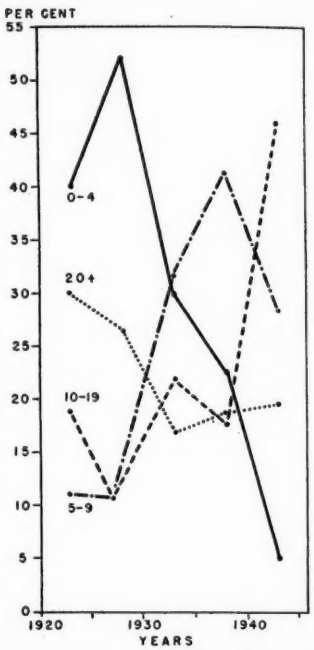

FIG. 2. Percentage distribution of age-specific mortality rates for poliomyelitis in Connecticut, 1921-1945. Legend as in Figure 1.

Table 5 illustrates the ratio of age-specific rates of the 0-4 group to the adjusted rate of all ages for all cases and fatal cases. Here again there is a relative decrease in incidence of poliomyelitis among those in the youngest age group. The tendency seems to be equally apparent in both the series of fatal cases and the total cases as indicated by the decreasing ratios over the successive periods of time.

Mention has also been made of one notable variable, namely, that the age incidence of poliomyelitis is related to population density. A number of analyses were made of this aspect of the situation in Connecticut. From them it was concluded that there is a tendency for younger cases to be relatively more numerous in the urban areas and the older cases relatively more numerous in the rural areas. But in general the data were not adequate for interpretation.

It is thus our impression that throughout the state of Connecticut the differences in the average age at which members of rural and urban populations contract poliomyelitis have never been very appreciable and are being largely erased.

Discussion

We come now to the question as to whether the trend indicated by the data from Connecticut on the age incidence of poliomyelitis is significant, and if so, what can be made of it. The author believes that it *is* significant, that a trend away from the infantile disease is apparent, but that its

TABLE 5

THE RATIO OF AGE-SPECIFIC RATES OF THE 0-4 GROUP TO THE ADJUSTED RATE OF ALL AGES FOR ALL CASES AND FATAL CASES

ALL CASES

Year	*Mean annual age-specific case rate, 0-4*	*Mean annual adjusted case rate, all ages*	*Ratio**
1916	386.0	65.0	5.9
1921-1925	27.0	4.9	5.5
1926-1930	21.2	4.7	4.5
1931-1935	88.6	22.8	3.9
1936-1940	7.4	2.5	3.0
1941-1945	39.4	14.8	2.7

FATAL CASES

Year	*Mean annual age-specific death rate, 0-4*	*Mean annual adjusted death rate, all ages*	*Ratio**
1916	96.0	17.7	5.7
1921-1925	5.6	1.4	4.0
1926-1930	3.1	0.6	5.2
1931-1935	4.6	1.6	2.9
1936-1940	0.6	0.3	2.0
1941-1945	0.4	0.6	0.7

* This ratio is the quotient of age-specific case (and mortality) rate of the 0-4 age group, divided by the adjusted case (and mortality) rate for all ages.

explanation is complicated and probably obscure. It cannot be explained entirely on the basis of shifts in age distribution of the population of Connecticut which have taken place during the past twenty-five years, or of differing diagnostic criteria used for the recognition of poliomyelitis during that period. The reasons for not accepting the first explanation have been given. The reasons for not accepting the second need further discussion, for it is difficult to determine how much the diagnostic criteria

have changed during the past quarter century. There is little doubt that abortive poliomyelitis is today diagnosed more frequently than it was prior to 1930. In the epidemic of 1916 in New York, for instance, the non-paralytic cases amounted to about 13 per cent of the total.[5] In 1935, in that same city, the non-paralytic cases were reported as 33 per cent of the total.[22] But it is not clear what the situation is today in this age when careful muscle testing tends to increase the relative number of paralytic cases. Many cases that might have been called non-paralytic a decade ago are now classified as paralytic—the new criteria for paralysis being transient weakness of a few days' duration.*

There has also been evidence that non-paralytic poliomyelitis is more common in the older age groups. Wickman[25] pointed out in 1913 that Müller made mention of the fact that non-paralytic cases were more numerous than the typical cases, especially among adults, and the same point was made in Denmark some twenty-five years later.[8] In 1933, Trask and his co-workers[18] showed that the paralyzed cases were more concentrated in the younger age groups than were the abortive cases and suggested that "there is a relationship between age and ability to abort the disease." That observation was made more than fifteen years ago and on the basis of 1932 diagnostic criteria, but the recent evidence is not yet available as to whether non-paralytic cases are today universally more common than are paralytic cases in the older age groups.

However, the answer to this problem could perhaps be achieved if only paralyzed cases were included in a series of this sort. From such a series in Massachusetts, Horton and Rubenstein[10] claim that the relationship between the morbidity rates of paralyzed cases in the whole population and the rates of paralyzed cases in the group under five years has remained relatively constant during the period 1910-1940, and that the relative decrease in similar ratios for total cases is therefore due to the increased reporting of abortive (a non-paralytic) poliomyelitis.

With the *fatal* cases this difficulty as to type of case of poliomyelitis should not arise, a point which has been made by Gilliam.[7] Therefore, an

* The diagnostic criteria used by the Yale Poliomyelitis Study Unit for the identification of *abortive poliomyelitis* are those of a disease which occurs coincidently with paralytic poliomyelitis but in which there are no clinical evidences of meningo-encephalomyelitic envolvement. The symptoms of this illness have been described by Paul, Salinger, and Trask[17] and by Horstmann and Paul.[9] The diagnostic criteria for *non-paralytic poliomyelitis* are those of an illness with clinical evidences of meningo-encephalomyelitic envolvement but in which paralysis or muscle weakness is not apparent ten days after the onset of second phase symptoms. Different criteria are probably used elsewhere.

evaluation of changes in mortality rates should not be affected by an increased tendency to report non-paralytic cases. It should show the same general trends as would a study of paralyzed cases. Yet the relation between the mortality rates for poliomyelitis in the whole population and the group under five, as indicated in Table 5, is not constant, and decreases over the period of time studied in the same way that the similar ratios for morbidity rates decrease. This observation, in addition to the fact that there has been a decline in morbidity since 1921 in the age-specific mortality rates of the 0-4 age group (shown in Table 4), would seem to refute Horton and Rubenstein's premise[10] that the apparent variation in the age distribution of poliomyelitis has been due almost entirely to a change in the age distribution of the population and an increase in the reporting of non-paralytic cases. We may conclude, rather, that in Connecticut the data suggest a real tendency for the disease to affect the younger population less than formerly and this is due to reasons which are as yet unexplained. Theoretically these reasons could be associated with the average size of families—which has changed. They could be related to changes in the environmental (or living) conditions which have altered rapidly over the years regardless of whether or not such changes could be considered as improvements in sanitation. Or they could be related to many other features too numerous to mention here. Nevertheless it would seem important to continue to seek out these reasons. For whatever factor or factors are responsible for the change in the age incidence of poliomyelitis, they have some bearing on the etiology or epidemiology of this disease and as such may be of some moment in considerations of its control.

Summary

The age distribution of cases of poliomyelitis in Connecticut have been studied chronologically over the period 1921-1947.

There has been a marked change in the proportionate age incidence of poliomyelitis in this state since 1921, with a relative decrease in the percentage of the cases in the 0-4 age group. In general, therefore, the disease no longer deserves the term "infantile paralysis" in this state.

Fatal cases have shown the same percentage age distribution as have the total cases as determined by the adjusted number of deaths derived from age-specific mortality rates.

It is concluded that these trends towards the "aging" of poliomyelitis patients cannot be directly explained merely on the basis of the "aging" of the population nor by the increased reporting of non-paralytic cases. Such trends are probably due to factors of which we are not yet fully informed.

References

1 Bertenius, B. S.: On problem of poliomyelitis; epidemiological-statistical study. Acta path. microb. scand., 1947, Suppl. 68, pp. 1-212.

2 Burnet, F. M.: The epidemiology of poliomyelitis, with special reference to the Victorian epidemic of 1937-38. J. Australia, 1940, *1*, 325. See also Poliomyelitis, Chapter V in *Virus as organism.* Cambridge, Harvard University Press, 1945, 134 pp.

3 Caverly, C. S.: Notes of an epidemic of acute anterior poliomyelitis. J. Am. M. Ass., 1896, *26*, 1.

4 Dauer, C. C.: Trends in age distribution of poliomyelitis in the United States. Am. J. Hyg., 1948, *48*, 133.

5 Fischer, A. E. and Stillerman, M.: Acute anterior poliomyelitis in New York in 1935; review of 686 cases. Am. J. Dis. Child., 1937, *54*, 984.

6 Forsbeck, F. C. and Luther, E. H.: Anterior poliomyelitis in Massachusetts, 1907-1929. N. England J. Med., 1930, *203*, 1115.

7 Gilliam, A. G.: Changes in age selection of fatal poliomyelitis. Pub. Health Rep., Wash., 1948, *63*, 677.

8 Henningsen, E. J. and Rasch, G.: The poliomyelitis epidemic of 1937 in Denmark; epidemiology and clinical statistics. J. Hyg., Lond., 1940, *40*, 84.

9 Horstmann, D. M. and Paul, J. R.: Incubation period in human poliomyelitis and its implications. J. Am. M. Ass., 1947, *135*, 11.

10 Horton, R. J. M. and Rubenstein, A. D.: The age distribution of poliomyelitis in Massachusetts. N. England J. M., 1948, *239*, 169.

11 Knowlton, Millard: Poliomyelitis in 1931. Connecticut Health Bull., 1932, *46*, 3.

12 Lavinder, C. H., Freeman, A. W., and Frost, W. H.: Epidemiologic studies of poliomyelitis in New York City and the northeastern United States during the year 1916. Pub. Health Bull., Wash., 1918, No. 91, pp. 185-187.

13 Macdonald, E. J.: The meaning of adjusted rates for cancer. Connecticut Health Bull., 1946, *60*, 279.

14 Nicoll, Matthias, Jr.: Epidemiologic data in the poliomyelitis epidemic in New York State. Am. J. Dis. Child., 1917, *14*, 69.

15 Paul, J. R.: Peripheral spread of poliomyelitis through rural and urban areas; observations from recent epidemic. Yale J. Biol., 1947, *19*, 533.

16 Paul, J. R.: Changing aspects in poliomyelitis. Tr. Ass. Am. Physicians, 1948, *61*, 301.

17 Paul, J. R., Salinger, R., and Trask, J. D.: "Abortive" poliomyelitis. J. Am. M. Ass., 1932, *98*, 2262. See also: Studies on the epidemiology of poliomyelitis; methods and criteria for the detection of abortive poliomyelitis. Am. J. Hyg., 1933, *17*, 587.

18 Paul, J. R., Salinger, R., and Trask, J. D.: Studies on the epidemiology of poliomyelitis; the incidence of abortive types of poliomyelitis. Am. J. Hyg., 1933, *17*, 601.

19 Starr, M. A.: Poliomyelitis anterior acute in *Albutt's System of Medicine.* London, Macmillan Co., 1899. pp. 198-199 in Vol. 7.

20 Wells, M. W.: Epidemiology, Chapter VII in *Poliomyelitis.* Baltimore, Williams & Wilkins Co., 1932. 562 pp.

21 Wells, M. W.: Cf. ref 20, p. 157.

22 Wenner, H. A.: Comparative statistical studies of epidemics of poliomyelitis in and about the City of New Haven, 1916-1943. Yale J. Biol., 1947, *19*, 331.

23 Wickman, Ivar: *Acute poliomyelitis.* Nervous and Mental Disease Monograph Series No. 16, 1913. 135 pp.

24 Windorfer, A.: Vom Wandel der Poliomyelitis. Zbl. inn. Med., 1942, *63*, 897.

ENDOCRINE REGULATION OF AMINO ACID AND PROTEIN METABOLISM DURING FASTING*

HENRY D. HOBERMAN

The concept of the dynamic state of the body constituents is basic to an understanding not only of those processes which maintain the constancy of body composition, but also of those which are concerned with the phenomenon of growth. It is evident from the work of Schoenheimer[23] and his colleagues that the amount of protein in the animal body is determined by the rates of protein synthesis and breakdown which prevail. From this it follows that growth, which is associated with the accumulation of body protein, can occur only when the rates of metabolism of amino acids and proteins are so adjusted as to favor an increase in the amount of body protein. Thus accumulation of protein might result from a retardation of amino acid or protein catabolism, or by speeding up of the synthesis of protein, or conceivably, by all mechanisms operating simultaneously. It may readily be seen that the control of each of these processes by independent mechanisms would afford the animal a smooth means of regulation of growth. If the immature animal accumulates protein by retarding catabolic reactions, it is, of course, necessary to assume that in the adult these processes proceed at rates faster than during growth. On the other hand, if it is assumed that growth occurs as a result of the acceleration of protein synthesis, it must also be assumed that the rate of synthesis of protein declines as the animal matures. Expressed in a different way, these considerations imply that those agencies which regulate the rates of amino acid and protein metabolism are functions of the age of the animal, and in view of the remarkable effects of endocrine secretions on growth, it follows logically that the factors which regulate the rates of metabolism of amino acids and body proteins of the living animal are endocrine in nature.

Theoretical considerations

The interrelationships of amino acid and protein metabolism may, perhaps, best be illustrated by means of the scheme below.

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$$\text{Body proteins} \rightleftarrows \text{Amino acids} \rightarrow \text{Urea}$$
$$\uparrow$$
$$\text{Diet}$$

Since it is possible that amino acid and protein catabolism and protein synthesis are each equally likely to respond to changes in the endocrine environment, it is indeed difficult to relate the activity of a particular hormone specifically to a change in the rate of any one of these processes. A complete kinetic treatment of this problem would probably include such factors as amino acid and protein inhomogeneity, the possibility of multi-order reactions, etc. However, such an analysis presents formidable obstacles in the way of mathematical expression. Thus in order to find solutions which might reasonably describe the state of amino acids and body protein in the living animal, it has been found expedient to make assumptions and approximations which not only yield mathematical expressions of some simplicity, but which are, at the same time, susceptible to experimental test. Whether or not the assumptions so made are consistent with reality can best be determined by comparing the results which are obtained by these means with those which have been achieved by the use of other experimental approaches.

In the analysis which follows it is assumed that insofar as their utilization for synthesis and catabolism is concerned, the amino acids and body proteins constitute two separate homogeneous reservoirs of nitrogen. It is also assumed that the rate at which nitrogen moves out of a particular reservoir is proportional to the amount of nitrogen in that reservoir. In the absence of dietary nitrogen all of the amino acids of the animal are derived from body protein, and the concept of the dynamic state may be expressed in the form of the following equations:

$$\text{(1)} \qquad \mathrm{P} \underset{k_2}{\overset{k_3}{\rightleftarrows}} \mathrm{A} \xrightarrow{k_1} \text{Urinary nitrogen}$$

$$\text{(2)} \qquad d\mathrm{A}/dt = k_3\mathrm{P} - (k_1 + k_2)\mathrm{A}$$

$$\text{(3)} \qquad d\mathrm{P}/dt = k_2\mathrm{A} - k_3\mathrm{P}$$

where P and A are, respectively, the amounts of protein and amino acid nitrogen in the dynamic state; k_1 is the fraction of A which, per unit time, t, is catabolized and excreted; k_2 is the fraction of A which, per unit time, is transformed into P; and k_3 is the fraction of P which, per unit time, is transformed into A.

The general solutions of (2) and (3) are:

$$\text{(4)} \qquad \mathrm{A} = me^{r_1 t} + ne^{r_2 t}$$

$$\text{(5)} \qquad \mathrm{P} = Me^{r_1 t} + Ne^{r_2 t}$$

where m, n, M, and N are constants of integration, and r_1 and r_2 are, respectively, $\dfrac{-(\beta + k_3) + \sqrt{(\beta + k_3)^2 - 4k_1k_3}}{2}$ and $\dfrac{-(\beta + k_3) - \sqrt{(\beta + k_3)^2 - 4k_1k_3}}{2}$, where $\beta = k_1 + k_2$. The roots, r_1 and r_2, may be simplified by binomial expansion of the radicals. The elimination of quadratic terms yields, approximately, $r_1 = -(\beta + k_3) - \lambda$,

$r_2 = -\lambda$, where $\lambda = \frac{k_1 k_3}{\beta + k_3}$. It will presently be shown that β is approximately 10^{-1} per hour, whereas k_3 and λ are, respectively, approximately 10^{-2} per hour, and 10^{-3} per hour. Considering the relative magnitudes of β, k_3, and λ, it is evident that with increasing time, the exponential in $r_1 t$ decreases at a rate much faster than that in $r_2 t$, so that at the given time, t_4, (4) and (5) become

(6) $$A_4 = ne^{-\lambda t_4}$$

(7) $$P_4 = Ne^{-\lambda t_4}$$

where t_4 is chosen sufficiently large to cause the exponential in $r_1 t_4$ to vanish, and A_4 and P_4 are the respective values of A and P at time, t_4. At any time, T, greater than t_4, (6) and (7) become

(8) $$A_T = A_4 e^{-\lambda T}$$

(9) $$P_T = P_4 e^{-\lambda T}$$

where t_4 is zero with respect to T, and the subscript, T, denotes the values of A and P at time, T. Thus, after a fast of t_4 hours, the loss of amino acid and protein nitrogen is represented as a process of first-order decay. The amount of amino acid and protein nitrogen lost during this decline may be seen to depend not only upon the extent of protein breakdown (k_3), but also upon the fraction of amino acids formed from protein which is catabolized and excreted (approximately equal to $k_1/(\beta + k_3)$). Thus, for example, it might be anticipated that a hormone which accelerates the catabolism of amino acids, but which does not influence protein breakdown, will nevertheless accelerate the loss of body protein. Measurements of protein loss during fasting cannot, therefore, make clear the distinction between the processes of amino acid and protein breakdown.

A quantitative relationship between A and P may now be derived. Rewriting (2) to account for the distinction between t and T yields

(10) $$dA_T/dT = k_3 P_T - \beta A_T$$

Differentiating (8) and substituting the value of dA_T/dT so obtained into (10) gives

(11) $$(\beta - \lambda) A_4 = k_3 P_4$$

or since $\beta >> \lambda$, approximately

(12) $$\beta A_4 = k_3 P_4.$$

Thus the foregoing treatment of the dynamic state leads to the conclusion that after fasting for t_4 hours, the amount of amino acid nitrogen which enters the amino acid reservoir ($k_3 P_4$) is approximately equal to the amount of nitrogen which leaves (βA_4).

The rate of nitrogen excretion is evidently

(13) $$dN/dt = k_1 A$$

where dN/dt is the rate of excretion of the nitrogen produced by the catabolism of A. From the relations expressed in (8) and (13) it may be anticipated that after a preliminary fast of t_4 hours, the rate of excretion of urinary nitrogen will begin to decline exponentially at the rate, λ. That this conclusion has a basis in fact is indicated by the common observation that after the first day, the nitrogen output of fasting rats falls so slowly that it is difficult to detect day-to-day differences. With λ of the order of 10^{-3} per hour, the expected daily fall in the rate of nitrogen excretion is approximately 2%. This is a difference which under ordinary conditions is difficult to demonstrate, since the number of animals required to provide data of statistical significance is unusually large.

A method for the measurement of k_1, k_2, and k_3 would, of course, provide the data necessary to relate the activity of a particular hormone specifically to the rate processes of amino acid and protein metabolism. Approximate values of k_1, k_2, and k_3P_4 can be calculated from data obtained by the use of isotopic nitrogen (N^{15}). In addition, the effect of a given hormone on the value of k_3 alone can be estimated by taking into account the changes in the amount of body protein which follow alterations in the endocrine environment. It is therefore possible to describe in quantitative terms the effects of various hormones on each of the rate processes.

When glycine containing an excess of N^{15} is administered to fasting rats, the excretion of isotopic nitrogen as urinary urea is as represented in the graph in Figure 1. Here the fraction of the administered dose which is excreted is plotted against the time. It may be seen that after the first 48 hours, the rate of excretion of N^{15} becomes exceedingly slow. The portion of the curve to the left of the broken line has been shown by Rittenberg[18] to fit the empirical equation

$$x = p(1 - e^{-qt}) \tag{14}$$

where x is the fraction of the administered isotopic nitrogen excreted in t hours, and q and p are undetermined constants. The portion of the curve to the right of the broken line may be shown approximately to fit an expression of the same general form as (14). Whereas the value of q calculated from (14) is approximately 10^{-1} per hour, the corresponding value of the rate constant which determines the slope of the flat portion of the curve is approximately 10^{-3} per hour. It is clear that the steep portion of the curve is determined by the rate of catabolism of the administered amino acid, and by the rate of its utilization for the synthesis of protein. The flat portion of the curve must represent the excretion of N^{15} derived from the breakdown of labelled body protein.

Recently Sprinson and Rittenberg[23] have shown that (14) is equivalent to

$$x = \frac{U}{U+S}\left(1 - e^{\frac{-(U+S)t}{A}}\right) \tag{15}$$

where x is as defined above, U is the amount of urinary nitrogen excreted per unit time, t, S is the amount of amino acid nitrogen which, per unit time, is incorporated into protein, and A is the amount of nitrogen in the amino acid reservoir. It is consistent with the assumptions mentioned in the mathematical analysis of the dynamic state that (15) is also equivalent to

$$E = \frac{k_1 a}{\beta}(1 - e^{-\beta t}) \tag{16}$$

where k_1 and β are as previously defined, E is the amount of N^{15} excreted in t hours, and a is the dose of administered N^{15}. It should be noted that neither k_3 nor P appear in any of the isotope equations. This omission is justified on the grounds that the rate at which isotopic nitrogen is contributed to the amino acid reservoir by the breakdown of labelled protein is small as compared with the rates of utilization of the administered amino acid.

Equation (16) is used to calculate k_1 and k_3 as follows: In this equation the exponential in $-\beta t$ may be made negligibly small by assigning a large value to t. For example, when $t = 48$ hours, and $\beta = 0.10$ per hour, $e^{-4.8} = 0.008$, which for practical purposes may be considered zero. If the total amount of N^{15} excreted during this time is E_t,
(16) is reduced approximately to

(17) $$E_t = k_1 a/\beta$$

and since a is known, k_1/β is readily calculated. Substitution of (17) into (16) yields

(18) $$E = E_t(1 - e^{-\beta t})$$

which when converted into the logarithmic form is

(19) $$\beta = \frac{2.3}{t} \log \frac{E_t}{E_t - E}$$

Thus by measuring the amounts of isotopic nitrogen excreted at times, t, and t_t, (17) and (18) or (19) may be solved. The values of k_1/β and β provide the information necessary to calculate k_1 and k_2. Equation (15) may be used to calculate the value of A by measuring, in addition to k_1, the rate of nitrogen excretion.

The use of the foregoing method is not restricted to the fasting state. When such a condition is imposed, however, the relationship expressd in (12) may be used to calculate the rate of protein breakdown (k_3P_t). The analysis of the dynamic state during fasting suggests that after one or two days, the amount of nitrogen which leaves the amino acid reservoir (βA_t) is approximately equal to the amount which enters (k_3P_t). If, after such a fast, a small dose of an isotopic amino acid (containing a mg. of N^{15}) is administered, the system may be considered to remain undisturbed, i.e., the amino acid reservoir is not significantly expanded by the amount of nitrogen given.* It may readily be shown, using the relations already expressed, that for the conditions outlined above, the rate of protein breakdown may be calculated from the formula

(20) $$k_3P_t = 100a/C_tT_t$$

where C_t is the isotope concentration (atom per cent excess) of the urinary urea which has been collected for T_t hours, and T_t is the time corresponding to t_t in obtaining Equation (17).

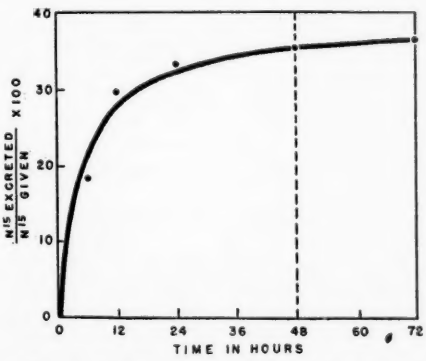

FIG. 1. Urinary excretion of N^{15} after the administration of glycine to normal fasting rats.

It has been a common observation in this laboratory that the nitrogen output of fasting rats falls exceedingly slowly from the second to as long as the fifth day of fasting. For this reason it has been the practice in carrying out the experiments described in this report to administer isotopic nitrogen (as N^{15} glycine) to rats after a preliminary fast of 24 hours. By this procedure the requirement demanded by Equations (6) and (7) is nearly quantitatively fulfilled.

The curve in Figure 1 shows that the excretion of N^{15} is an exceedingly rapid process during the first 24 hours. For this reason it is important that collections of urine are started as soon after the admin-

* In the experiments described in this report, the amount of nitrogen in the isotopic glycine administered was equivalent to 2% of the amino acid nitrogen content of the animals.

istration of the labelled amino acid as is practicable. It is evident that accurate calculations can be made only when the differences between E and E_4 are significant. Although it would be desirable to obtain frequent samples of urine, experience in this laboratory indicates that collections of urine 10 and 48 hours after the injection of isotopic glycine are most compatible with practicability. Samples obtained at some reasonable interval after 10 hours already contain amounts of isotopic nitrogen which preclude use in evaluating Equations (18) or (19). Collections made at some time before 10 hours are likely to suffer from inaccuracy.

The values of E_4, E, and k_3P_4 have been calculated from the isotope concentration of the urinary total nitrogen rather than from the isotope concentration of the urea. However, the isotope concentration of the urea, as well as the amount of urea nitrogen, excreted by several animals in each group was measured in all experiments reported. The data obtained show that under whatever conditions the experiments were performed, the isotope concentration of the urea bore an almost constant relationship to that of the total nitrogen. The amount of N^{15} excreted as urea accounted for 90 to 100% of the isotope in the urinary total nitrogen.

Experimental

Male rats of the Sprague-Dawley strain, 13-15 weeks of age, were used. Adrenalectomy and thyro-parathyroidectomy were performed by the usual procedures. Hypophysectomized Sprague-Dawley rats were obtained from a commercial laboratory.* Diabetes was induced by the intravenous injection of alloxan in a dose of 40 mg. per kilogram of body weight. The fasting blood sugar and the extent of weight loss were the criteria used to establish the presence of diabetes.

Adrenalectomized animals were provided with drinking bottles containing 1% sodium chloride solution, and the thyro-parathyroidectomized rats with an aqueous solution containing 1.8% calcium lactate and 0.2% calcium chloride. All animals were fed Purina Fox Chow pellets preliminary to the experimental period.

Experiments were performed on adrenalectomized rats on the 6th postoperative day, on thyro-parathyroidectomized animals on the 30th postoperative day, and on hypophysectomized rats on the 28th postoperative day. Diabetic rats were used 14 days after the administration of alloxan.

The injection of isotopic glycine and the collection of urine were carried out in a dehumidified room maintained at approximately 25° C. All animals were fasted for 24 hours before the administration of N^{15} glycine and, unless otherwise stated, for 48 hours after the injection of glycine. One ml. of a 1% solution of isotopic glycine (32.2 atom per cent excess) was administered per 100 gm. of body weight by intraperitoneal injection. In order to assure the collection of adequate volumes of urine, 0.85% sodium chloride solution was injected intraperitoneally at 2-hour intervals during the first 10 hours of each experimental day. One ml. of this solution was administered per 100 gm. of body weight. Adrenalectomized rats received, in addition to saline, 0.25 mg. of desoxycorticosterone acetate† by subcutaneous injection on the morning of each day of fasting.

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* Hormone Assay Laboratories, Chicago, Illinois.

† Percorten, Ciba. Supplied through the courtesy of Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Inc.

Results

Nitrogen metabolism of fasting normal and adrenalectomized rats. In Tables 1 and 2 are recorded the data obtained when the rate of excretion of N^{15} was measured after the administration of isotopic glycine to fasting normal and adrenalectomized rats. In agreement with the reports of others[6, 8] it was found that the nitrogen output of fasting adrenalectomized rats is less than that of normal animals. It may be seen that this defect is

TABLE 1

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF NORMAL RATS

*Weight**	E_{10}	E_{48}	C_{48}	*Urinary N†*	k_1	k_2	k_2P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
300	0.410	0.669	0.223	2.08	3.48	6.01	5.70
340	0.412	0.685	0.199	2.08	3.04	6.16	6.40
330	0.379	0.613	0.195	2.00	2.94	6.68	6.52
310	0.352	0.610	0.209	1.97	2.77	5.83	6.09
316	0.396	0.654	0.199	2.16	3.11	6.19	6.40
328	0.500	0.745	0.200	2.37	4.13	6.97	6.36
Mean				**2.11**	**3.25**	**6.31**	**6.25**
S. E.‡				0.06	0.20	0.18	0.12

* Dose, $a = \frac{\text{weight (to nearest dekagram)} \times 0.61}{100}$

‡ Standard error $= \sqrt{\frac{1}{n(n-1)} \sum_{i=1}^{n} (x_i - \bar{x})^2}$

† Calculated from 48-hour nitrogen output.

not related to any detectable abnormality in the catabolism of amino acids. However, the amount of amino acid nitrogen formed from body protein is significantly depressed by adrenalectomy ($P < 1\%$), while at the same time the fraction of amino acid nitrogen used for the synthesis of protein (k_2) is greater than normal ($P < 2\%$).

Comparison of the body compositions of normal and adrenalectomized rats has shown that the protein content of the latter is proportionately greater than normal.[27] The slower rate of protein breakdown associated with a deficiency of adrenal hormones must therefore be ascribed to a decrease in the magnitude of the rate constant, k_3, denoting that the process

of conversion of body protein to amino acids is inhibited. This effect is sufficient almost quantitatively to account for the fall in the output of urinary nitrogen. To a lesser extent the acceleration of protein synthesis is also responsible for the lower rate of excretion of nitrogen.

Other investigators have reported that adrenalectomy may be responsible for a defect in the deamination of amino acids,[20, 21] or that the adrenal steroids specifically influence the rate of protein breakdown.[4] The stimulation of the process of protein formation, as observed in the present study,

TABLE 2

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF ADRENALECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
315	0.452	0.642	0.243	1.78	4.02	8.02	5.35
320	0.353	0.549	0.211	1.74	2.89	7.41	6.15
379	0.541	0.782	0.232	1.85	3.96	7.79	5.48
338	0.431	0.695	0.237	1.81	3.24	6.41	5.36
367	0.431	0.676	0.216	1.78	3.07	7.07	5.89
350	0.490	0.755	0.247	1.82	3.70	6.76	5.15
Mean				**1.80**	**3.48**	**7.24**	**5.56**
S. E.				0.02	0.20	0.25	0.15

is consistent with the observation of White, *et al.*,[20] who administered N^{15} glycine to fasting normal and adrenalectomized mice and found that the net uptake of N^{15} into the proteins of the internal organs, serum proteins, lymphatic tissue, and muscle was increased by adrenalectomy.

Insofar as the metabolism of nitrogen is concerned, removal of the adrenal glands appears chiefly to influence protein metabolism; the rate of incorporation of amino acids into protein increases, and the rate of protein breakdown falls.

Effects of cold on the nitrogen metabolism of fasting normal rats. It is well known that adrenal hypertrophy follows exposure to cold.[24] In order to study the changes in the metabolism of nitrogen which might be induced by overactivity of the adrenal glands, normal rats, each occupying a separate cage, were placed in a cold room at 4° C. Food and water were provided for 9 days. On the 10th day, while still in the cold room, the

animals were subjected to a fast of 24 hours, after which N^{15} glycine was administered. Fasting was continued, and the rate of excretion of N^{15} was measured. Of the 12 rats subjected to this procedure, 2 survived for more than 36 hours after the injection of isotopic glycine. The data obtained in this experiment are recorded in Table 3.

These results indicate that the catabolism of amino acids and body protein of normal fasting animals is considerably accelerated by severe cold, while the utilization of amino acids for protein synthesis declines. Consequent to these changes, the output of urinary nitrogen is strikingly increased.

TABLE 3

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF NORMAL RATS SUBJECTED TO COLD

Weight	E_{10}	E_{36}	C_{36}	*Urinary* N*	k_1	k_2	k_3P^4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
300	0.797	1.112	0.166	6.20	7.66	5.00	10.2
312	0.915	1.290	0.165	6.95	8.48	3.92	10.3

* Calculated from 36-hour nitrogen output.

At autopsy of 9 of the 12 animals the average weight of the adrenal glands was 73.6 ± 2.1 mg., as compared with 37.3 ± 3.5 mg. for 9 controls. The adrenal weights of the 2 surviving rats were 81.3 mg. and 65.3 mg. Involution of the thymus, spleen, and lymph nodes of the animals exposed to cold was notable.

Effects of thyro-parathyroidectomy. Data on the effects of thyro-parathyroidectomy on the nitrogen metabolism of fasting rats are summarized in Table 4. Comparison of these observations with those in Table 1 indicates that the catabolism of amino acids is significantly accelerated in the absence of the thyroid gland ($P < 1\%$), and that the rate of protein breakdown is less than normal ($P < 1\%$). In order to avoid the assumption that the body compositions of normal and thyro-parathyroidectomized rats are identical, the rates of protein breakdown are better compared when they are calculated for the whole animal, rather than per 100 gm. of body weight. When this is done it is found that the hypothyroid rats converted an average of 13.5 mg. of protein nitrogen to amino acid nitrogen, each hour, as compared with 20.1 mg. for the normal group, a decrease of 33%.

Addis, *et al.*,[1] who compared the protein contents of normal and thyroidectomized rats found that the latter contained only 17% less protein than normal. Thus it follows that in the absence of the thyroid hormone, the mechanisms which bring about the breakdown of body protein are operating at a lower level than normal.

Although the rate of protein breakdown is diminished by thyro-parathyroidectomy, the concomitant increase of amino acid catabolism appears to be sufficient to cause a slight increase over normal in the rate

TABLE 4

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF THYRO-PARATHYROIDECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
264	0.562	0.801	0.229	2.76	6.10	5.99	5.54
272	0.565	0.876	0.300	2.24	5.50	4.85	4.23
285	0.705	0.955	0.312	2.24	7.24	6.15	4.06
275	0.554	0.820	0.261	2.38	5.40	5.85	4.86
294	0.492	0.780	0.277	2.00	4.39	5.57	4.58
270	0.571	0.797	0.222	2.77	6.10	6.49	5.70
Mean				**2.40**	**5.79**	**5.82**	**4.83**
S. E.				0.13	0.39	0.23	0.23

of excretion of urinary nitrogen ($P < 5\%$). That the urea output of hypothyroid rats is not less than normal has also been reported by Persike,[15] and confirmed by Rupp, *et al.*[17] The latter also showed that the administration of small doses of thyroxin to thyroidectomized rats brought about the retention of nitrogen.

It will be noted that the rate of incorporation of amino acids into protein is not significantly different from normal.

Effects of adrenalectomy on the nitrogen metabolism of thyro-parathyroidectomized rats. The acceleration of amino acid catabolism exhibited by normal animals subjected to cold suggests that hyperactivity of the adrenal cortex may be responsible for the effects observed. Since thyro-parathyroidectomized animals also catabolize amino acids at a high level, the influence of adrenalectomy on the nitrogen metabolism of these animals was investigated. The data obtained from this study are shown in

Table 5. Removal of the adrenal glands did not bring about a decline in the value of k_1 in thyro-parathyroidectomized rats. It is also evident that adrenalectomy appears to be responsible for stimulation of protein synthesizing reactions whether performed on normal or hypothyroid rats. ($P < 1\%$).

Although amino acid catabolism is accelerated, the nitrogen output is significantly lower in the absence of both the adrenal and thyroid glands than after extirpation of either gland alone. The decrease in the excretion

TABLE 5

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF THYRO-PARATHYROIDECTOMIZED RATS AFTER ADRENALECTOMY

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
285	0.605	0.806	0.438	1.34	6.35	7.55	2.91
292	0.693	0.901	0.402	1.67	7.50	7.20	3.16
279	0.652	0.863	0.474	1.36	7.15	6.95	2.68
279	0.638	0.855	0.401	1.59	6.85	6.85	3.16
280	0.562	0.752	0.583	0.96	6.07	7.63	2.18
297	0.615	0.862	0.385	1.57	5.89	6.61	3.29
Mean				**1.41**	**6.63**	**7.13**	**2.90**
S. E.				0.11	0.26	0.16	0.17

of nitrogen may readily be accounted for by the conspicuous fall in the rate of conversion of body protein to amino acids.

Effects of hypophysectomy and of the administration of adrenocorticotrophic hormone to hypophysectomized rats. Evidence has been presented above to show that a high rate of amino acid catabolism is related to adrenal hyperactivity. The adrenal steroids also seem to influence the metabolism of protein by increasing the rate of protein breakdown and decreasing the utilization of amino acids for protein synthesis. In order to obtain information relating the adrenal glands more specifically to these processes, a study was made of the effects of adrenocorticotrophic hormone (ACTH) on the nitrogen metabolism of fasting hypophysectomized rats. Two-tenths (0.2) ml. of a 0.5% solution of ACTH* in isotonic saline was administered by intraperitoneal injection to hypophysectomized rats 4

* Kindly made available by Armour and Co., Chicago, Illinois.

times daily for 7 days. Each rat thus received 4 mg. of the hormone each day. On the 6th day of treatment, isotopic glycine was administered, and the rate of excretion of N^{15} was measured. The results obtained with the

TABLE 6

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF HYPOPHYSECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_2P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
159	0.336	0.446	0.321	1.82	6.40	7.60	3.96
159	0.332	0.437	0.297	1.92	6.35	7.85	3.83
163	0.310	0.446	0.258	2.20	5.45	6.45	4.93
164	0.326	0.470	0.258	2.31	5.73	6.07	4.93
156	0.274	0.386	0.293	1.76	4.92	7.48	4.34
158	0.291	0.433	0.256	2.23	4.95	6.15	4.97
Mean				**2.04**	**5.63**	**6.93**	**4.50**
S. E.				0.10	0.27	0.33	0.21

TABLE 7

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF HYPOPHYSECTOMIZED RATS RECEIVING ACTH

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_2P_4
			atom per cent	*mg. per 100 gm.*			*mg. per 100 gm.*
gm.	*mg.*	*mg.*	*excess*	*per hour*	*per cent per hour*	*per cent per hour*	*per hour*
153	0.328	0.490	0.233	2.86	5.93	5.17	5.44
151	0.329	0.517	0.225	3.18	5.72	4.38	5.65
148	0.394	0.571	0.212	3.80	7.34	4.36	6.00
152	0.325	0.473	0.221	2.94	6.02	5.58	5.75
142	0.292	0.449	0.204	3.23	5.55	4.95	6.22
132	0.269	0.422	0.247	2.71	5.42	4.68	5.14
Mean				**3.12**	**6.00**	**4.85**	**5.70**
S. E.				0.05	0.28	0.19	0.05

untreated and treated hypophysectomized rats are given in Tables 6 and 7. These data show that the catabolism of amino acids in fasting hypophysectomized rats is significantly greater than normal ($P < 1\%$), and

that this process was not measurably influenced by ACTH. On the other hand, the rate of protein catabolism, which is depressed in the absence of the pituitary gland, is notably increased ($P < 1\%$), while the value of k_2 is significantly diminished by the treatment ($P < 1\%$). It is interesting that the value of k_2 tends to be greater than normal in hypophysectomized rats ($P < 5\%$).

Under the conditions employed in these experiments, the nitrogen output of hypophysectomized rats does not appear to be different from normal.

TABLE 8

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF HYPOPHYSECTOMIZED RATS RECEIVING GROWTH HORMONE

Weight	E_{10}	E_{48}	C_{48}	*Urinary N*	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*			*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*			*per cent per hour*	*per cent per hour*	
172	0.222	0.361	0.301	1.45	3.34	6.21	4.22
154	0.251	0.346	0.349	1.34	4.91	7.99	3.64
163	0.281	0.368	0.337	1.39	5.45	8.95	3.76
158	0.181	0.268	0.276	1.28	3.02	8.18	4.60
180	0.197	0.312	0.254	1.42	2.95	7.03	5.01
158	0.151	0.253	0.247	1.30	2.36	6.72	5.14
Mean				**1.36**	**3.67**	**7.51**	**4.40**
S. E.				0.03	0.44	0.42	0.26

The explanation of this observation in terms of the rates of nitrogen metabolism is similar to that offered in the case of the thyro-parathyroidectomized animals, i.e., the acceleration of amino acid catabolism compensates for the fall in protein catabolism. The rise in the output of nitrogen following treatment with ACTH appears to be a consequence of a more rapid rate of protein breakdown and of a decline in the rate of incorporation of amino acids into protein.

Effects of growth hormone on the nitrogen metabolism of hypophysectomized rats. It is well known that the administration of anterior pituitary extracts or growth hormone either to normal or hypophysectomized rats is followed by a decrease in the output of urinary nitrogen. In order to relate this effect to specific processes involved in the metabolism of

* Supplied through the courtesy of Dr. A. E. Wilhelmi, Department of Physiological Chemistry, Yale University School of Medicine. The growth hormone was estimated to be 95% homogeneous by electrophoresis.

amino acids and body protein, growth hormone was administered to hypophysectomized rats, and a study made of the rate of excretion of N^{15} after the injection of isotopic glycine. Two-tenths ml. of a 0.5% solution of crystalline growth hormone* in isotonic saline was administered by intraperitoneal injection to hypophysectomized rats 3 times daily for 3 days. Each rat thus received 3 mg. of the hormone each day. On the second day of treatment isotopic glycine was injected, and the rate of excretion of N^{15} was measured in the usual manner.

TABLE 9

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF THYRO-PARATHYROIDECTOMIZED RATS RECEIVING GROWTH HORMONE

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
255	0.392	0.560	0.269	1.77	4.25	7.75	4.93
260	0.188	0.339	0.217	1.31	1.73	6.35	6.12
240	0.392	0.549	0.287	1.73	4.69	7.81	3.49
250	0.359	0.506	0.265	1.66	4.18	8.42	5.00
260	0.342	0.522	0.247	1.76	3.50	7.10	5.36
260	0.363	0.561	0.289	1.62	3.69	6.71	4.59
Mean				**1.64**	**3.67**	**7.36**	**4.91**
S. E.				0.07	0.42	0.31	0.36

Comparison of the data in Tables 6 and 8 show that growth hormone reduced the value of k_1 to a normal level ($P < 1\%$). A similar effect of growth hormone on the catabolism of amino acids has been reported by Russell and Cappiello,[19] who observed that the accumulation of urea in the blood of nephrectomized rats receiving a mixture of amino acids is depressed by growth hormone.

It will be noted that growth hormone did not appear to influence the rate of protein breakdown or the rate of utilization of amino acids for the synthesis of protein.

Effect of growth hormone on the nitrogen metabolism of thyro-parathyroidectomized rats. The data of Table 5 would seem to exclude the possibility that the acceleration of amino acid catabolism in hypothyroidism is due to hyperactivity of the adrenal cortex. Thus it may be that either the synthesis or secretion of growth hormone is depressed in the absence of the

thyroid gland, or that the peripheral activity of growth hormone depends upon thyroxin for its expression. To distinguish between these mechanisms, growth hormone* was administered to thyro-parathyroidectomized rats. The dosage used, and the schedule of injections were as described earlier. The data of Table 9 show that growth hormone does indeed repress the catabolism of amino acids in the absence of the thyroid gland ($P < 1\%$). On the other hand, the data indicate that the treatment did not evoke a change in the rate of protein breakdown. It should be noted, however, that the rate of incorporation of amino acids into protein was significantly increased by the hormone ($P < 1\%$).

It has already been shown that removal of the adrenal glands brings about a profound fall in the rate of protein breakdown of thyro-parathyroidectomized rats. The failure of growth hormone to produce a similar effect in hypothyroid animals is therefore consistent with the hypothesis that the effects of growth hormone on the metabolism of protein are not due to a direct repression of the activity of the adrenal cortex or to the peripheral inhibition of the protein catabolic effects of the adrenal steroids.

Effect of growth hormone on the nitrogen metabolism of adrenalectomized rats. The experiments described earlier in this report show that although growth hormone inhibits the catabolism of amino acids in the absence of the hypophysis or thyroid gland, no significant changes in the metabolism of protein were detected in the absence of the pituitary gland. On the other hand, it has been shown that growth hormone does accelerate the synthesis of protein in thyro-parathyroidectomized animals. Since the activity of the adrenal cortex is low in hypophysectomized animals, the possibility must be considered that growth hormone does not influence protein synthesis in the absence of the adrenal cortex. In order to test this hypothesis, growth hormone, in the dosage already mentioned, was administered to adrenalectomized rats. The data in Table 9 show that growth hormone did not influence protein metabolism in these animals. On the other hand it may be seen that a significant fall in the rate of catabolism of amino acids occurred ($P < 1\%$), suggesting that the effect of growth hormone on amino acid catabolism is independent of the adrenal cortex.

It has previously been reported that the nitrogen output of adrenalectomized animals is lowered by the administration of anterior pituitary extracts.[9] It may be noted in Table 10 that the nitrogen output of growth hormone-treated adrenalectomized rats is also less than that observed in

* Supplied by Armour and Co. through the kindness of Dr. Irby Bunding.

untreated controls ($P < 1\%$). This may be accounted for entirely in the depression of amino acid catabolism resulting from the treatment.

The data in Tables 9 and 10 indicate that the synthesis of protein is accelerated by growth hormone only in the presence of the adrenal cortex. It has already been suggested that growth hormone does not influence either amino acid or protein metabolism by repressing the activity of the adrenal cortex. Nevertheless, it would appear that adrenal steroids must be present in order that growth hormone may increase the rate of utilization of

TABLE 10

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF ADRENALECTOMIZED RATS RECEIVING GROWTH HORMONE

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_2P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
310	0.296	0.490	0.198	1.70	2.40	6.86	6.56
315	0.358	0.522	0.238	1.48	3.10	8.50	5.46
285	0.286	0.482	0.226	1.59	2.46	6.54	5.74
270	0.258	0.462	0.243	1.50	2.30	5.87	5.35
293	0.275	0.481	0.267	1.31	2.30	6.17	4.86
Mean				**1.52**	**2.51**	**6.79**	**5.59**
S. E.				0.06	0.15	0.46	0.28

amino acids for the synthesis of protein. It has further been suggested that growth hormone does not influence protein metabolism by the peripheral inhibition of the protein catabolic effect of the adrenal steroids. It would therefore seem clear that growth hormone must influence protein synthesis by peripheral inhibition of that effect of the adrenal hormones which specifically inhibits protein synthesis.

Nitrogen metabolism in alloxan-diabetes. Previous investigators have suggested that the nitrogen-sparing action of anterior pituitary extracts is exerted through effects on the islets of the pancreas.[14] Since insulin has been shown to inhibit the accumulation of amino nitrogen in the blood of eviscerated rats,[7, 18] it might be anticipated that in diabetes the rate of incorporation of amino acids into protein is slower than normal. In Table 11 are recorded the data obtained when the rate of excretion of N^{15} was studied after the administration of N^{15} glycine to alloxan-diabetic rats. These data show that in diabetes the catabolism of amino acids and body

protein is significantly accelerated. Attention should also be directed at the value of the rate constant, k_2, which is lower than any value previously observed. It will be noted that the depression of protein synthesis and the acceleration of protein breakdown are similar to those effects resulting from the administration of ACTH to hypophysectomized rats. Thus it is difficult in this experiment to distinguish between effects which on the one hand might be due to the absence of insulin, and on the other, to the presence of a high concentration of adrenal steroids. For this reason N^{15} glycine was

TABLE 11

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF ALLOXAN-DIABETIC RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary N*	k_1	k_2	k_2P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
190	0.507	0.824	0.139	6.50	6.80	2.74	9.14
195	0.586	0.953	0.145	7.03	7.43	2.09	8.95
233	0.516	0.874	0.156	5.11	5.56	3.38	8.34
252	0.581	0.900	0.167	4.55	6.10	4.20	7.77
Mean				**5.80**	**6.47**	**3.10**	**8.55**
S. E.				0.58	0.41	0.45	0.31

administered to diabetic animals from which the adrenal glands had been extirpated. It may be seen in Table 12 that after removal of the adrenal glands from diabetic animals, the rapid breakdown of amino acids and body protein ceases, and, indeed, is reduced to the same levels as previously described for adrenalectomized-non-diabetic animals. However, the rate of utilization of amino acids for the synthesis of protein remains depressed, indicating that it is the absence of insulin, rather than adrenal hyperactivity, which is directly responsible for the low rate of protein synthesis. This suggests that the adrenal steroids inhibit the utilization of amino acids in anabolic reactions by bringing about the inactivation of insulin, or by otherwise nullifying the peripheral effects of insulin. This view is supported by Ingle's[10] observation that in diabetic rats, tolerance to insulin is markedly increased by the administration of 17-hydroxy-11-dehydrocorticosterone (Kendall's Compound E).

Influence of Compound E on the nitrogen metabolism of adrenalectomized and adrenalectomized-thyro-parathyroidectomized rats. Data have been

presented which indicate that both the adrenal cortex and the thyroid gland influence the rate of conversion of body protein to amino acids. It seemed of interest to determine to what degree the hormones of the adrenal cortex influence the breakdown of body protein in the absence of the thyroid gland. For this purpose a suspension of Compound E in peanut oil was prepared in a concentration of 8 mg. per ml. Two mg. of Compound E (0.25 ml. of the suspension) were injected subcutaneously into adrenalectomized and adrenalectomized-thyro-parathyroidectomized rats two hours before the

TABLE 12

URINARY EXCRETION OF N^{15} AND RATES OF AMINO ACID AND PROTEIN METABOLISM OF ALLOXAN-DIABETIC RATS AFTER ADRENALECTOMY

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
240	0.380	0.689	0.266	2.84	3.77	4.23	4.78
240	0.378	0.698	0.235	2.63	3.72	4.08	5.52
210	0.176	0.500	0.237	2.09	1.67	2.61	5.48
240	0.341	0.656	0.246	2.32	3.29	4.06	5.16
Mean				**2.47**	**3.11**	**3.75**	**5.24**
S. E.				0.17	0.49	0.38	0.17

administration of isotopic glycine, and again 10 hours after the injection of isotope. These doses were again administered on the following day. The data in Tables 13 and 14 show that Compound E accelerates the catabolism of amino acids in adrenalectomized rats ($P < 1\%$), and also significantly raises the level of amino acid catabolism in adrenalectomized-thyro-parathyroidectomized rats ($P < 1\%$). It is evident that the treatment also caused a marked increase in the rate of protein breakdown in the adrenalectomized animals ($P < 1\%$), and almost doubled the rate of protein breakdown in the adrenalectomized-thyro-parathyroidectomized rats. Although the rate of protein catabolism induced by the treatment tends to be less marked in the doubly operated group, the hormone tended to increase the rate of protein catabolism in these animals above that observed in untreated thyro-parathyroidectomized rats ($P < 5\%$). It will also be noted that in both groups of animals the values of k_2 are significantly less than in the untreated controls ($P < 1\%$, in each instance). It is of considerable

TABLE 13

EFFECT OF COMPOUND E ON THE NITROGEN METABOLISM OF ADRENALECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
288	0.548	0.819	0.168	3.52	5.12	5.92	7.56
297	0.554	0.820	0.165	3.48	5.04	6.21	7.70
340	0.678	0.975	0.212	2.82	5.59	6.31	6.00
350	0.716	1.172	0.172	4.06	5.17	4.25	7.38
302	0.651	0.990	0.194	3.52	5.79	4.91	6.55
338	0.502	0.896	0.212	2.61	3.56	4.66	6.00
Mean				**3.34**	**5.05**	**5.38**	**6.87**
S. E.				0.22	0.32	0.36	0.32

TABLE 14

EFFECT OF COMPOUND E ON THE NITROGEN METABOLISM OF ADRENALECTOMIZED-THYRO-PARATHYROIDECTOMIZED RATS

Weight	E_{10}	E_{48}	C_{48}	*Urinary* N	k_1	k_2	k_3P_4
			atom per cent excess	*mg. per 100 gm. per hour*	*per cent per hour*	*per cent per hour*	*mg. per 100 gm. per hour*
gm.	*mg.*	*mg.*					
265	0.835	1.11	0.249	3.50	9.39	4.56	5.10
240	0.726	1.05	0.213	4.29	8.40	3.29	5.96
240	0.721	1.01	0.228	3.84	8.62	3.88	5.58
245	0.710	1.08	0.215	4.26	7.59	3.16	5.91
Mean				**3.97**	**8.50**	**3.72**	**5.64**
S. E.				0.19	0.31	0.33	0.20

interest that this effect is significantly more pronounced in the hypothyroid animals ($P < 1\%$).

Discussion

The results of the experiments described in this report provide additional evidence to support the view that the metabolism of amino acids and body proteins is significantly influenced by secretions of the endocrine glands.

Endocrine regulation of amino acid catabolism. The data which have

been presented show that in fasting rats the catabolism of amino acids is accelerated by adrenal cortical steroids and is depressed by the pituitary growth hormone. Since the depression of the catabolism of amino acids by growth hormone is observed in adrenalectomized animals, it may be concluded that in the intact animal this effect is not produced by repression of the activity of the adrenal cortex.

The acceleration of amino acid breakdown which follows thyro-parathyroidectomy would also seem to be independent of the function of the adrenal cortex. This suggests that in hypothyroidism the rapidity of breakdown of amino acids is due to a release of the catabolizing system from the inhibitory influence of growth hormone. The observation that in hypophysectomized rats the catabolism of amino acids proceeds at approximately the same rate as in thyro-parathyroidectomized animals is consistent with this view. The marked effect of Compound E in accelerating the catabolism of amino acids of adrenalectomized-thyro-parathyroidectomized rats therefore favors the concept that in the intact animal the cortical steroids do not exert a direct effect on the activity of growth hormone. It would follow from the above that ACTH should accelerate the catabolism of amino acids when administered to hypophysectomized rats. This was not observed in the experiments reported here. However, it should be pointed out that the effect of the adrenal cortex on the catabolism of amino acids does not appear to become manifest until the level of cortical activity is greater than normal. It is therefore possible that under the conditions of the experiment referred to above, the required activity of the adrenal cortex was not obtained.

The question might well be raised as to the nature of the mechanism which, in the absence of the thyroid gland, leads to a decline in the activity or amount of growth hormone. The data in Table 9 would suggest that the peripheral activity of growth hormone does not depend upon the presence of thyroxin, for a significant depression of amino acid breakdown occurred when growth hormone was administered to thyro-parathyroidectomized rats. This indicates that the secretion or synthesis of growth hormone is strongly influenced by the activity of the thyroid gland. The fact that growth hormone depressed the level of amino acid catabolism in hypophysectomized animals is also consistent with this view. It has been reported,[8] however, that the treatment of hypophysectomized rats with anterior pituitary extracts and thyroxin results in a more rapid rate of growth than when either of these preparations is administered separately, suggesting that thyroxin potentiates the activity of growth hormone.

Whether or not the growth-promoting effects achieved by the combination of these hormones is entirely related to the repression of amino acid catabolism cannot be stated at this time.

The data obtained from diabetic animals would seem to exclude the possibility that the inhibition of amino acid catabolism induced by growth hormone is exerted by an effect on the pancreas. Although the catabolism of amino acids is considerably accelerated in fasting diabetic animals, the data in Table 12 show that after adrenalectomy, the level of amino acid catabolism is restored to normal. This interpretation is consistent with the observations of Russell and Cappiello,[19] who showed that in nephrectomized rats, insulin does not reproduce the effect of growth hormone in depressing the rate of formation of urea from administered amino acids. It has long been suggested[13] that the alleviation of diabetes induced by adrenalectomy might in part be explained as being due to a depression of amino acid and protein catabolism, with a concomitant fall in the rate of gluconeogenesis. The results of the experiments reported in the present communication provide additional evidence to support this view.

Experiments (unpublished) recently performed in this laboratory with isotopic nitrogen show that in fasting rats the conversion of ammonia to urea is far too rapid to offer the possibility that the hormonal regulation of amino acid catabolism is concerned with the operation of the Krebs-Henseleit cycle. Moreover, when ammonium citrate, labelled with N^{15}, was administered to fasting hypophysectomized rats, the rate of formation of isotopic urea was not lowered by growth hormone. Thus it may be that in the fasting intact animal the rate of urea formation is determined by the rate of deamination of amino acids. It is on this process that the influence of growth hormone and the adrenal steroids would seem to be exerted.

It follows from the foregoing considerations that during fasting the intact animal possesses two independent means for the hormonal regulation of amino acid catabolism. During growth the retention of nitrogen resulting from a diminution in the catabolism of amino acids might conceivably be brought about either by the secretion of "extra" growth hormone, or by the suppression of adrenal cortical activity. The first of these two possible mechanisms is more consistent with the evidence presented in this report, for in the adult animal the catabolism of amino acids does not appear to be depressed by adrenalectomy. This may also be interpreted to indicate that in the absence of stress the adrenal cortex has but little quantitative influence on the catabolism of amino acids.

Hormonal regulation of protein breakdown. The experiments presented

in this communication indicate that the rate of conversion of body protein to amino acids is increased by the adrenal cortical steroids (e.g. Compound E), and is depressed in the absence of the adrenal, thyroid, or pituitary glands. Removal of both adrenal and thyroid glands brings a decline in the rate of protein breakdown which is greater than when either of these organs is removed separately. This suggests either that the thyroid and adrenal hormones operate in synergy to mobilize amino acids from body protein, or that the proteins susceptible to Compound E-induced breakdown are qualitatively different from those whose destruction is induced by the activity of the thyroid gland.

White and Dougherty,[85] who studied the effects of endocrine secretions on the loss of protein during fasting, have suggested that the mobilization of nitrogen from the carcasses of fasting mice is under thyroid control, whereas the breakdown of the proteins of lymphatic tissue is directly regulated by the adrenal hormones. They have also suggested that by indirect action, i.e., via the thyroid gland, the adrenal steroids may also bring about the breakdown of carcass protein. They suggest further that the adrenal and thyroid hormones may also exert a synergistic effect on protein catabolism.

It will be recalled (Tables 13 and 14) that the administration of Compound E to adrenalectomized-thyro-parathyroidectomized rats causes a striking acceleration of protein breakdown. It can be calculated from the amount of nitrogen excreted by these animals that in two days, protein-containing tissue* equivalent to 8% of the body weight must have been destroyed. This would seem to be somewhat in excess of that which might be estimated to arise from non-carcass sources and suggests that the proteins of the rat carcass are indeed sensitive to the catabolic effects of the cortical hormones whether or not the thyroid gland is present.

An estimation of the amount of body protein which is readily available for conversion to amino acids provides additional information relative to the hormonal regulation of protein catabolism.

The amount of body protein which is susceptible to breakdown during fasting is defined in Equation (11). In the logarithmic form this expression is

(21)† $$\lambda = \frac{2.3}{T} \log \frac{P_i}{P}$$

* Assumed to contain 15% protein.

† Although as derived, the use of this equation is limited to the period of starvation following a preliminary fast of one or two days, theoretical considerations indicate that little error is introduced into the calculations which follow when this condition is not met.

Since P is the amount of protein remaining in the animal after a fast of T days, (21) may also be written

$$\lambda = \frac{2.3}{T} \log \frac{P_i}{P_i - y} \tag{22}$$

where y is the amount of protein lost during the fast. Rewriting (22) as follows

$$\frac{k_1 k_3}{\beta + k_3} = \frac{2.3}{T} \log \frac{k_3 P_i / k_3}{k_3 P_i / k_3 - y} \tag{23}$$

yields an equation which may be solved for k_3 if the values of k_1, k_2, $k_3 P_i$, and y are known.

Addis, Poo, and Lew[2] have reported that in 7 days the average amount of protein nitrogen lost by normal fasting rats is 296 mg. per 100 gm. of body weight. With y thus equal to 296 mg., $k_1 = 0.0325$ per hour, $k_2 = 0.0631$ per hour, and $k_3 P_i = 6.25$ mg. per 100 gm. per hour, (Table 1); the value of k_3 obtained by evaluation of these data in (23) is 4.9×10^{-3} per hour. The values of λ and P_i are thus 1.6×10^{-3} per hour, and 1.28 gm. per 100 gm. respectively. The total amount of body protein in the rats employed by Addis, *et al.*, was equivalent to 2.74 gm. of nitrogen per 100 gm. of body weight. Thus 1.28/2.74, or approximately 47% of the total protein of the fasting rat is estimated to be susceptible to breakdown at the rate, k_3. The remaining 53%, it must be assumed, yields its amino acids at a much slower rate. On this basis the degree of lability of a tissue, measured in terms of its protein content, would be proportional to the amount of protein in that tissue in the more labile state. Thus the difference in apparent labilities of the total protein contents of the various tissues and organs would largely disappear. For example, from the data of White and Dougherty, it can be calculated that if 50% of the carcass protein of the mouse is susceptible to rapid breakdown, 25% of this labile protein would be lost during a fast of 48 hours. Assuming that 100% of the protein of lymphatic tissues is similarly labile, 31% would be lost from these animals in the same period. Thus the point may be made that measurements of protein loss during fasting would not seem to provide evidence sufficiently definitive to show that the protein content of an individual organ is specifically influenced by a hormone. It is more consistent with present evidence that the effect of hormones which induce the acceleration of protein catabolism express their activities in a non-specific manner. It may also be tentatively assumed that the thyroid hormone potentiates the protein-catabolic effects of the adrenal cortical steroids.

Hormonal regulation of protein synthesis. The results of the experiments presented in this report show that the biochemical reactions leading to the formation of protein are accelerated in adrenalectomized and hypo-

physectomized rats, and, when the adrenal glands are intact, in animals treated with growth hormone. On the other hand, the synthesis of body protein is diminished in those animals in which the adrenal cortex is hyperactive, or, when the adrenal glands are removed, in diabetic animals. It is clear from these observations and from the work of others that insulin is required in order for the synthesis of protein to take place at normal or accelerated rates. It would therefore appear that the acceleration of the conversion of amino acids to protein in adrenalectomized and hypophysectomized rats is to be attributed to an increase in the effective concentration of insulin, a view which is consistent with the fact that adrenalectomized and hypophysectomized animals are hypersensitive to insulin.

The fact that ACTH brings about a depression of protein formation in hypophysectomized rats indicates that insofar as the metabolism of protein is concerned, insulin, rather than growth hormone, is the target at which the activity of the adrenal cortical hormones is directed. Recent data (unpublished) obtained in this laboratory show that in hypophysectomized rats the depression of protein synthesis resulting from the administration of ACTH is partially reversed by growth hormone. On the other hand it was found that growth hormone did not inhibit the protein-catabolic effects of ACTH. Earlier in this report it was pointed out that growth hormone does not repress the breakdown of protein in hypophysectomized and in thyroparathyroidectomized rats. Similar effects (unpublished) have recently been observed when growth hormone was administered to normal animals. Although the synthesis of protein was increased by the treatment, no depression of protein catabolism was observed. The available evidence is therefore consistent with the hypothesis that growth hormone accelerates the synthesis of protein by inhibiting the adrenal-induced inactivation of insulin, or by inhibiting those effects of the adrenal cortical steroids which oppose the peripheral effects of insulin.

The data in Table 14 show that in hypothyroidism, Compound E induces a greater diminution in the rate of conversion of amino acids to protein than when the thyroid gland is present. This may be interpreted as indicating that in the absence of thyroxin the anabolic effects of insulin are more readily influenced by adrenal cortical activity, suggesting that the thyroid gland is concerned with the regulation of the secretion or synthesis of growth hormone. It may be recalled that a similar suggestion was offered to explain the effects of hypothyroidism on the catabolism of amino acids. Although it might be anticipated that the rate of conversion of amino acids

to protein would be less than normal in hypothyroidism, the data in Table 4 do not support this expectation. However, these data would be consistent with the foregoing hypothesis if it can be demonstrated that the activity of the adrenal cortex is depressed in the absence of the thyroid gland.

The biochemical reactions which are so modified by insulin as to bring about a faster rate of utilization of amino acids for the synthesis of protein have not been identified. Charalampous and Hegsted[3] have recently reported that the acetylation of p-aminobenzoic acid is depressed in diabetic rats and suggest that a primary deficiency of adenosine triphosphate (ATP) is responsible for this effect. This hypothesis is supported by the observation of Kaplan and Greenberg[11] that an increase in the uptake of P^{32} into liver ATP can be demonstrated after the administration of insulin. If the formation of peptide bonds occurs in a manner analogous to the acetylation of amines, other evidence[19] would seem to indicate that the depression of protein synthesis observed in diabetic rats may also be assumed to be due to a deficiency in ATP. In any event the relationship of insulin to energy-yielding processes would seem to support the hypothesis that in the living animal, the rate of utilization of amino acids for the synthesis of protein is determined by the rates of those reactions which supply the energy required for the formation of peptide bonds. It would seem to be consistent with this that the major effects of those hormones which influence the synthesis of protein are exerted on those reactions which yield the energy required for the synthesis of peptide bonds.

It is evident that the rate of protein formation can be limited not only by the rate at which energy is provided, but also by such important and variable elements as the availability of amino acids, the concentrations of energy-yielding substrates, the amounts and activities of the enzymes which direct syntheses, etc. In addition, there are factors which must determine the qualitative properties of protein structures, i.e., which direct the synthesis of a particular "kind" of protein. The results of the present study do not at all exclude the possibility that hormones may indeed specifically influence any one or all of these factors. They do demonstrate, however, that the endocrine secretions strongly influence the rate of utilization of amino acids for the formation of protein through their influence on energy-yielding reactions.

ACKNOWLEDGMENTS

The author is indebted to Mr. Joseph Doolittle, Mr. Jack Graff, Dr. Bernard Kartin, Miss Florence Kee, Mr. David Stone, and Miss Dorothy von Hacht for their capable assistance.

References

1 Addis, T., Karnofsky, D., Lew, W., and Poo, L. J.: The quantities of protein lost by the various organs and tissues of the body during a fast. J. Biol. Chem., 1938, *124,* 33.

2 Addis, T., Poo, L. J., and Lew, W.: The quantities of protein lost by the various organs and tissues of the body during a fast. J. Biol. Chem., 1936, *115,* 111.

3 Charalampous, F. C. and Hegsted, D. M.: Acetylation in the diabetic rat. J. Biol. Chem., 1949, *180,* 623.

4 Engel, F. L., Schiller, S., and Pentz, E. I.: Studies on the nature of the protein catabolic response to adrenal cortical extract. Endocrinology, 1949, *44,* 458.

5 Evans, G. T.: The adrenal cortex and endogenous carbohydrate formation. Am. J. Physiol., 1936, *114,* 297.

6 Evans, H. M., Pencharz, R. I., and Simpson, M. E.: On the conditions necessary for the continous growth of hypophysectomized animals. Endocrinology, 1935, *19,* 509.

7 Frame, E. G. and Russell, J. A.: The effects of insulin and anterior pituitary extract on the blood amino nitrogen in eviscerated rats. Endocrinology, 1946, *39,* 420.

8 Harrison, H. E. and Long, C. N. H.: Effects of anterior pituitary extracts in the fasted rat. Endocrinology, 1940, *26,* 971.

9 Harrison, H. E. and Long, C. N. H.: The effect of anterior pituitary extract on the metabolism of fasting normal and adrenalectomized rats. Am. J. Physiol., 1939, *126,* 526.

10 Ingle, D.: The physiological action of the adrenal hormones in: *The chemistry and physiology of the hormones,* 1944, A.A.A.S., Washington, D. C.

11 Kaplan, N. O. and Greenberg, D. M.: Studies with radioactive phosphorus of the changes in the acid-soluble phosphates in the liver coincident to alterations in carbohydrate metabolism. J. Biol. Chem., 1944, *156,* 525.

12 Lipmann, F.: Mechanism of peptide bond formation. Fed. Proc., Balt., 1949, *8,* 597.

13 Long, C. N. H. and Lukens, F. D. W.: The effects of adrenalectomy and hypophysectomy upon experimental diabetes in the cat. J. Exp. M., 1936, *63,* 465.

14 Mirsky, I. A.: The influence of insulin on the protein metabolism of nephrectomized dogs. Am. J. Physiol., 1938, *124,* 569.

15 Persike, E. E.: Increased protein catabolism in thyroidectomized rats. Rates of urine urea excretion and serum urea concentrations. Endocrinology, 1948, *42,* 356.

16 Rittenberg, D.: The application of the isotope technique to the study of the metabolism of glycine. Biol. Sympos., Lanc., 1948, *13,* 173.

17 Rupp, J., Paschkis, K. E., and Cantarow, A.: Influence of thyroxin on protein metabolism. Endocrinology, 1949, *44,* 449.

18 Russell, J. A. and Cappiello, M.: The relationship of temperature and insulin dosage to the rise in plasma amino nitrogen in the eviscerated rat. Endocrinology, 1949, *44,* 127.

19 Russell, J. A. and Cappiello, M.: The effects of pituitary growth hormone on the metabolism of administered amino acids in nephrectomized rats. Endocrinology, 1949, *44,* 333.

20 Russell, J. A. and Wilhelmi, A. E.: Metabolism of kidney tissue in the adrenalectomized rat. J. Biol. Chem., 1941, *137,* 713.

21 Samuels, L. T., Butts, J. S., Schott, H. F., and Ball, H. A.: Glycogen formation after alanine administration in adrenalectomized animals. Proc. Soc. Exp. Biol., N. Y., 1937, *35,* 538.

22 Schoenheimer, R.: *The dynamic state of body constituents.* Cambridge, Mass., Harvard University Press, 1942.

23 Sprinson, D. and Rittenberg, D.: The rates of interaction of the amino acids of the diet with the tissue proteins. J. Biol. Chem., 1949, *180,* 715.

24 Tepperman, J. and Engel, F. L.: *Metabolic determinants of adrenal size and function.* Josiah Macy Foundation, New York, 1942.

25 White, A. and Dougherty, T. F.: Role of the adrenal cortex and the thyroid in the mobilization of nitrogen from the tissues in fasting. Endocrinology, 1947, *41,* 230.

26 White, A., Hoberman, H. D., and Szego, C. M.: Influence of adrenalectomy and fasting on the incorporation of isotopic nitrogen into the tissues of mice. J. Biol. Chem., 1948, *174,* 1049.

27 Winternitz, J.: *The compositions of the gains in weight of adrenalectomized rats.* Yale University. Thesis, part II, 1942.

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ARTHUR HENRY MORSE

1880 - 1950

Dr. Arthur Henry Morse died on January 26, 1950 at the age of 69. He is survived by his wife and two sons. After graduation from Tufts College in 1902 and the Johns Hopkins Medical School in 1906, he served as resident in pathology at the Rhode Island General Hospital. Subsequently, in 1910, he became resident and instructor in obstetrics at Johns Hopkins Hospital and in 1912 resident surgeon at the Union Protestant Infirmary (Baltimore). From 1913 to 1915 he was instructor in obstetrics and gynecology at the University of California and associate gynecologist at the University Hospital (San Francisco). He came to the Yale University School of Medicine in 1915 as assistant professor of obstetrics and gynecology. He became professor of obstetrics and gynecology and chairman of the department in 1921, a position which he held until his retirement as emeritus professor in 1948. He belonged to the American Gynecological Society, the New England Surgical Society, was a fellow of the American College of Surgeons and the American Medical Association. He was one of the charter members of the American Board of Obstetrics and Gynecology and before his retirement he was obstetrician- and gynecologist-in-chief of the New Haven Hospital and New Haven Dispensary. This is a short review of his medical career.

On the occasion of Dr. Morse's retirement from the Chair of Obstetrics and Gynecology the following minute was presented to the Board of Permanent Officers of the School of Medicine, Yale University:

Dr. Arthur Morse retires this year as Professor of Obstetrics and Gynecology in the Medical School bearing the love and respect of his colleagues. It would be difficult in the long history of the School of Medicine to find a member of the faculty who gave more unselfishly and generously of his talents to furthering the highest interests of the institution and its affiliated hospital and to developing to an admirable degree the discipline in which he was specially interested. The Board of Permanent Officers wishes to record in its minutes the gratitude of Yale University to Dr. Morse for his many years of loyal and effective service.

The Board of Permanent Officers wishes to salute Dr. Morse for his accomplishments as a teacher, recognizing as it does that many leading practitioners of Obstetrics and Gynecology in City, State, and Nation are indebted to him for skillful guidance in their development in their special field of work. Three of his former pupils have occupied full professorships in other medical schools and many other pupils trained by Dr. Morse fill important academic posts of lesser rank.

Dr. Morse's work in the field of gynecological pathology has brought him recognition as an authority. Some years ago, with Doctors van Wagenen and Zuckerman as associates, he began the study of the physiology of reproduction in the monkey. The results of this fundamental research are recognized today as authoritative in the field of primate studies in reproduction. Carried into the clinical field these investigations

have advanced our knowledge of the pathology of pregnancy and clarified problems associated with infertility.

Fortunately for the School of Medicine, Dr. Morse's official retirement does not terminate his interest and influence in the institution which he has unobtrusively but devotedly and effectively served since he came to Yale in 1915. His colleagues associate themselves with pupils and friends in extending good wishes for the future and gratitude for a life of fine integrity, unselfish loyalties, and generous and rewarding service to patients, students, School, and profession.

In addition to Dr. Morse's interest in research was his devotion to teaching. He was a fine teacher, unsparing of himself in time and effort. No part of his medical life gave him greater satisfaction. This interest in serving others extended far beyond his curricular duties and many of our younger physicians will recall with deep gratitude the many extra hours which he gave to them. Because he had the fine qualities of a natural teacher, medical students and others continously sought his counsel and advice. In such conference they were made confident by his sure insight, deep interest, and unfailing kindness.

In his contact with patients as a clinician he displayed an elevation of manner combined with kindness and sympathy which is recognized as greatness in medicine. In this he reflected the worth and teaching of those leaders under whom he had trained. They lived in him.

Words such as these cannot express the individual sense of loss which his passing will mean to those who were fortunately numbered among his friends. His was a generous friendship which forgets self. He looked always for the best in his friends and by that very act called forth the best from them. They saw in him that which Sir Thomas Browne extoled as nobility without heraldry and a natural dignity.

No true picture of Dr. Morse can be drawn without expressing his appreciation of humor as a part of the experience of life. The keenness of this sense was often seen not only in the quiet and very appropriate remark, but on occasion as a short and pointed poem with which to delight his intimates. This sense also served to carry him through the inevitable periods of stress which come to all men of important position. To him controversy in the usual sense was belittling and usually served no useful purpose. His opinions, however, lacked no firmness and he was unswerving whenever rightness was called to question.

To a friend he wrote, "I have two loyalties, one to my family, the second to the clinic." His devotion to his family, like his other devotions, is unassessable. A certain joy of living came to him through family life, the memory of which will remain a treasure to those who shared it with him. To his friends this is also a precious memorial, for they saw in this devotion its rare worth.

The words of the Connecticut poet will never be truer spoken than to the memory of Arthur Morse,

"None knew thee but to love thee,
Nor named thee but to praise."

HERBERT THOMS

NEWS AND COMMENT

ON YALE RESEARCH REPORTED IN OTHER JOURNALS

Testosterone and growth. The phenomenon of growth now holds the interest of investigators in many different disciplines, one of which is endocrinology. The anterior lobe of the pituitary, for example, has been for decades regarded as a regulator of growth, and study of its action has now been made easier through the discovery of an improved method of preparation of the "growth" hormone.* Not only at Yale, but in several laboratories elsewhere, actions of this hormone and its influence upon the intermediary metabolism of protein, carbohydrate, and fat are being investigated.

Another hormone now believed to have a growth promoting action is testosterone. Its activity has been studied especially in rats, and claims for its ability to bring about growth have been questioned and disputed by some. This lack of agreement, however, only adds to the significance of a recent account of the use of testosterone in two young female monkeys, where growth and development were greatly enhanced.† The hormone was injected (intramuscularly in vegetable oil) from the age of 5 months, in a dose of 7.5 mg./kg./week. Seven months later, at an age of one year, the monkeys had reached the weight and body length of normal two-year-old monkeys, and began to menstruate like two-year-olds.

Control data for the experiment were taken from records of animals in the colony maintained through five generations in the Department of Obstetrics and Gynecology. In particular, figures for weight, length, and date of menarche of twenty young females recently raised in the colony were employed. The two treated monkeys apparently reached true sexual maturity, with the bodily size of young adults, at an age of one year or just one year earlier than their colony mates. At present, the mechanism of the early development is unknown; other glands, including the pituitary, may well be involved. Further studies are in progress.

A pump for by-passing the right heart. The success with which surgeons are now correcting certain anomalies of blood vessels within the thorax has created new interest in the possibility of operating upon the heart. To this end, a variety of pumps have been devised which circulate the blood while the heart is temporarily at rest, and various means of by-passing the lungs, too, have been proposed. Like many other forms of treatment, the clinical use of these pumps and oxygenators requires their perfection, and a clear demonstration of their successful use upon laboratory animals.

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* Fishman, J. B., Wilhelmi, A. E., and Russell, Jane A.: A crystalline pituitary protein with high growth activity. Science, 1947, *106*, 402.

† van Wagenen, Gertrude: Accelerated growth with sexual precocity in female monkeys receiving testosterone propionate. Endocrinology, 1949, *45*, 544.

In a recent report from the Department of Surgery a pump of simple construction is described, together with an account of its utilization in dogs to by-pass the right heart and to maintain the lesser circulation for periods as long as 100 minutes.* The pump consists of a reinforced tube made of thin rubber, mounted within a glass cylinder where negative and positive pressures may be alternately applied around the rubber tube. Valves direct the flow of blood as the negative pressure first draws blood into the pump from a cannula in the great veins, following which the positive pressure forces the blood on into a cannula in one of the pulmonary arteries. The pulsatile character of both the venous drainage and the arterial supply appears to be an important factor in the success of the pump. Both in principle and in design the apparatus is uncomplicated—a great asset in any piece of equipment. Its usefulness, at least in experimental animals, may well be limited only by the surgical skill necessary to place it in the circulation, or to restore the normal circulation when the pump is removed.

Like the heart-lung preparation described by H. Newell Martin and later utilized by Starling and others, this pump substitutes a mechanical device for a part of the circulatory system. In the heart-lung preparation the mechanical part is the systemic circulation, where peripheral resistance, venous pressure, and the temperature of the blood may be altered at will to study their influence upon arterial pressure, stroke volume, and heart rate. The system carries on without nervous or humoral regulation. With the right heart pump, on the other hand, the peripheral parts of both circulations are intact, and the possibility of their regulation by other parts of the body is preserved. Heart rate, stroke, and minute volume are the variables which may be manipulated; the influence of their varying upon other parts of the system, upon blood flow, or upon the composition of the blood may be studied. By this technique the progress of physiology as well as that of experimental surgery will be aided.

Hormones and the genesis of tumors. What the rat and guinea pig are to nutrition, the mouse is to experimental studies of cancer. Numerous reports, one of which appears in this number of the *Journal,* testify to the usefulness of this small rodent in research into the genesis and growth of tumors. It may not be equally well known that in certain strains of mice, particular organ systems lend themselves especially well to this type of study. One such system is composed primarily of the endocrine glands, and secondarily of other organs such as the mammary glands and reproductive tract, the growth and development of which are dependent upon endocrine function. Thus, the induction of tumors in each of four glands of internal secretion has been described—namely, tumors of the testis in certain strains of mice injected with estrogen; of the anterior pituitary in other strains upon similar injection; in the adrenal cortex of castrated mice; and in ovaries

* Sewell, W. H., Jr. and Glenn, W. W. L.: Observation on the action of a pump designed to shunt the venous blood past the right heart directly into the pulmonary artery. Bull. Am. Coll. Surgeons, 1950, *35,* 53.

transplanted into the spleen or pancreas of castrates.* In every instance the development of the tumor is attributed to an over-abundance within the mouse's tissues of one or another hormone of the trophic type, which in itself is an entirely "normal" compound. In excess, its effects become abnormal. The conditions leading to the development of these tumors have been called "hormonal imbalance."

The endocrine glands seem to lend themselves to this study for two reasons. First of all, they normally show quite a little variability in their degree of differentiation, development, and function; morphologically, they are among the more labile tissues of the body. This is true also of the mammary glands and the female reproductive tract, which normally undergo marked changes in size, histological appearance, and activity. Second, many of the factors responsible for these changes have been discovered and made available for general laboratory use, including use by workers in the field of cancer research.

Observations of this nature have sometimes been interpreted to mean that hormones are the cause of cancer. Though this conclusion may be correct, at the present time it is unjustified, since closer analysis must lead only to the more restricted conclusion that hormones influence the development of cancer in tissues normally sensitive to them. If a general conclusion is to be stated, it can only be that under certain conditions, tumorigenesis is influenced by the factors which normally have a regulatory action upon the tissue in question.

J. R. B.

APPOINTMENT OF ASSOCIATE EDITORS

The Editors of *The Yale Journal of Biology and Medicine* are pleased to announce the appointment of the following as Associate Editors:

Robert Holmes and Raymond Rappaport, Jr., from the Graduate School; Ralph Gross, Ora Louise Kingsley, and John Frazier Snyder, from the School of Medicine.

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*Gardner, W. U.: Hormonal imbalances in tumorigenesis. Cancer Res., 1948, *8*, 397; cf. also a paper in press, *ibid.*

EVENTS

MICROBIOLOGY SEMINAR

January 13, 1950

MOULDS AS METABOLIC MODELS. By Jackson F. Foster, Department of Bacteriology, University of Texas, and Oak Ridge National Laboratories.

Under controlled laboratory conditions certain fungi are known to accumulate organic acids; the acid accumulated is dependent upon the substrate used. *Rhizopus nigricans,* which accumulates fumaric acid when grown on ethanol, was used to determine the validity of the Wood-Werkman reaction. Using labeled compounds it was found that the formation of a C_4 compound (i.e., fumaric acid) results from the condensation of two C_2 molecules and that carbon dioxide enters into the reaction only after prefixation. In the case studied the Wood-Werkman reaction is probably not the mode of genesis of the C_4 acids.

The same methods were utilized in the study of the formation of citric acid with the exception that *Aspergillis niger* was substituted for *R. nigricans.* In this case citric acid may be formed by the condensation of three C_2 molecules and not by the classical method ($C_1 + C_3 \rightarrow C_4 : C_4 + C_2 \rightarrow C_6$).

These studies seem to support the hypothesis put forward by Thunberg some twenty years ago; and suggest that the currently accepted Krebs cycle and connected schemes may be of less importance than is now generally assumed.

R. H.

ZOOLOGICAL JOURNAL CLUB

January 27, 1949

ECOLOGY OF PELAGIC *Daphnia.* By John Brooks, Instructor, Department of Zoology, Yale University.

In certain parthenogenetic fresh water forms, the body proportions of genetically identical generations have been found to show seasonal variation. This phenomenon has been termed cyclomorphosis. The discussion was confined to seasonal variation in relative length of the head in *Daphnia retrocurva.* Seasonal samplings of a population of *D. retrocurva* in Bantam Lake, Connecticut, showed that relative head length was at a minimum in early April but increased in successive generations to a maximum in late June and early July. Individuals with elongate heads and those with short heads showed the same vertical distribution patterns throughout this period, the greatest part of the population being confined to the upper strata of the lake. The general validity of Woltereck's hypothesis that relative head length determines distribution is thus brought into serious question. During

the summer the water in these upper strata becomes warm and turbulent. Culture experiments indicate that these environmental factors may be important determinants of relative growth.

R. R., JR.

February 1, 1950

ORGANIZATION OF THE TELEOST BLASTODERM. By Jane M. Oppenheimer, Professor of Biology, Bryn Mawr College.

Comparison of the eggs of the trout and the killifish, *Fundulus heteroclitus,* demonstrated that the slower development and higher degree of differentiation in the former at the time of closure of the blastopore can in great measure be correlated with its larger yolk content. In trout gastrulae, regions of the blastoderm explanted to yolk sac epithelium differentiate into characteristic tissue complexes. Repetition of this operation on *Fundulus* resulted only in differentiation of pigment and red blood cells. The opinion was expressed that these observations do not necessarily demonstrate a difference between the two forms as to degree of determination of the blastoderm, but may rather illustrate differences in external milieu required for cellular differentiation. An instance in *Fundulus* of differentiation of pronephros, from posterior blastoderm which had been transplanted to anterior blastoderm, to some extent supports this hypothesis. Thus the term "dependent differentiation" should be accompanied by information as to the factors upon which a differentiating part is dependent.

R. R., JR.

YALE MEDICAL SOCIETY

February 8, 1950

THE CLINICAL USES OF STREPTOKINASE-STREPTODORNASE. By William S. Tillett, Professor of Medicine, New York University College of Medicine.

The use of a streptococcal concentrate containing a fibrinolysin (Streptokinase) and a desoxyribonuclease (Streptodornase) was described in clinical conditions requiring the removal of fibrin and purulent exudate. Streptokinase acts as a kinase in the plasminogen-plasmin fibrinolytic system, and consequently requires the presence of plasminogen. Streptodornase produces liquefaction of pus by direct action on desoxyribonucleoprotein which comprises 70% of the solid sediment of purulent exudate. Treatment is by topical application.

A series of successfully treated cases was presented, including hemothorax, sterile and septic empyema, draining pleural sinus, purulent maxillary sinusitis, lung abscess and burns. In addition to facilitating drainage by lysis of pus and fibrin, the treatment in some cases permitted access of antibiotic and chemotherapeutic agents. Failure of treatment in some cases was attributed to the absence of plasminogen and to organization of fibrin. Toxic side effects consisted of a pyrogenic reaction with

leucocytosis, chills or chilly sensations, and arthralgias. Possible additional inapparent toxicities are now under investigation.

The potentialities of the enzyme approach to the removal of pathological tissue was discussed.

R. T. M.

PHARMACOLOGY SEMINAR

February 9, 1950

RECENT ADVANCES IN ANESTHESIA. By Wesley Bourne, Chairman, Department of Anesthesiology, McGill University.

A review of recent experimental work in the field of anesthesiology was prefaced by remarks on advances in education in this field.

The advantages and disadvantages of several synthetic relaxant drugs related to d-tubocurarine, with emphasis on decamethonium iodide ("C_{10}") and tolserol, were described. Attention was called to the recent observation that curare is useful in preventing a fall in blood pressure during surgical procedures carried out in the proximity of pressure receptors and nerves which reflexly control the heart rate, and further, the observation that coronary dilator drugs are useful for their anti-fibrillary action.

New anesthetic tools such as the oximeter and a plastic endotracheal tube for children with a non-rebreathing, nonresistant valve were described.

Recent theories on the mode of action of anesthetics in producing unconsciousness provided a provocative conclusion.

V. L. S.

BEAUMONT CLUB

February 17, 1950

BOYLE AND BAYLE, THE SCEPTICAL CHYMIST AND THE SCEPTICAL HISTORIAN. By Dr. George Sarton, Professor of the History of Science, Harvard University.

The second half of the seventeenth century was marked by an upsurgence of experimental science, philosophy, and the arts. The tremendous amount of intellectual activity during this period is not at all surprising when one considers the catalogue of great men of letters living at this time. From their intellectual keenness stemmed the development of the experimental method and the wisdom of a skeptical spirit and a rationalistic attitude towards the truths revealed by, and the conclusions drawn from, its use.

Two representative personages who lived during this half-century were Robert Boyle and Pierre Bayle, one a scientist, and the other an historian. They both crusaded against irrationalism, superstition, intolerance, and gullibility. Boyle struck out against the unscientific and superstitious literature of the alchemists by writing a book entitled *The Sceptical Chymist.* The book's approach is that of a discussion among proponents of the

Aristotelean, Paracelsian, and atomist schools of philosophy regarding the physical-chemical nature of matter. Although an atomist himself, Boyle did not propagandize for atomism because this theory had not yet been proved, thus preserving objectivity which runs as an undertone throughout the book. He felt, apparently, that once people really understood the many diverse views that existed, the logical outcome would be the development of a skeptical spirit and tolerance rooted in rationality.

While Boyle was carrying on his work in England, Bayle was in Holland concerning himself with religious intolerance. He came from a Protestant sect in France which had been persecuted by the Catholics of that country. Taking refuge in Holland, he met many Catholics there who, conversely, had been persecuted by the Protestants in England. From their and from his own experiences, Bayle developed a deep resentment toward religious intolerance and persecution. He subsequently wrote and spoke his conviction of the need for rationalism and tolerance as the common ground upon which men of diverse beliefs could meet. He championed the cause still further by being instrumental in the publication of a new journal, *The Republic of Letters,* the purpose of which was to bring learned men together and teach them to regard problems in an objective manner. He also wrote and published *The Dictionary Historical and Critical* for which he is probably known best. Bayle's apparent *modus vivendi* of *truth above all* is what prompted him to undertake this literary endeavor. The Dictionary is a compilation of historical and contemporary events written in an objective fashion which hitherto had not been done. Historians previous to Bayle were prone to let their personal views color their text, thus lending bias and obscuring the truth.

Boyle and Bayle, scientist and historian, each developed the skeptical spirit so necessary for the effective use of observation and experiment.

J. B. LER.

ANNUAL LECTURE
YALE CHAPTER OF ALPHA KAPPA KAPPA

February 24, 1950

RECENT ADVANCES IN MEDICINE. By Major General Raymond W. Bliss, Surgeon General, U.S. Army.

General Bliss cited briefly some of the more recent accomplishments made in medicine by the Army to illustrate the very significant role the Army has had in medical research.

Extensive investigations of the effects of radiation have shown that the cytoplasm, and perhaps more particularly certain enzyme systems within the cytoplasm, rather than the nucleus, is first affected. No significant increase in tolerance to radiation has been achieved to date by the use of drugs. Severe burns have been found to heal very well by using an undressed or open wound technique with early skin grafting. A rather

ingenious technique for following the progress of infection in burn cases and for determining the relative effectiveness of the various antibiotics was described. The drugs under question are contained in separate, insoluble tablets which can be embedded in blood agar media; the drugs diffuse into the media immediately around the tablets. The burn is swabbed and the agar plate streaked; the relative effectiveness of the different drugs in inhibiting growth is easily ascertained by this procedure.

Procaine-penicillin in the treatment of streptococcal upper respiratory infection has shown promising results in preventing any subsequent rheumatic fever. It has also been found that of the men followed after being rejected by the Army in World War II because they had hypertension at the time of examination, many now have no hypertension. Treatment of blood plasma with ultraviolet light has proved very effective in preventing homologous serum jaundice. A rat repellent called anti-dione is so effective that when offered no other alternatives, the rat will starve to death rather than contaminate himself with it to get at food.

The medical student is likely to view the science of medicine in terms of the specific medical problems which individual patients represent; though such an approach is wholly valid, General Bliss suggested that much can also be learned by a more impersonal or global approach. It is largely on the latter precepts that Army medical research is based, and the above illustrations of medical progress are ample indication of the importance and fruitfulness of the Army research program.

K. G. B.

BARRON, D. H.: CO_2 pressure gradient across the placenta. Fed. Proc., Balt., 1949, *8,* 8. From the Laboratory of Physiology.

BUNTING, HENRY: The histochemical detection of iron in tissues. Stain Techn., 1949, *24,* 109. From the Department of Pathology.

BUNTING, M. I., ROBINOW, C. F., AND BUNTING, HENRY: Factors affecting the elaboration of pigment and polysaccharide by Serratia marcescens. J. Bact., Balt., 1949, *58,* 114. From the Department of Pathology.

CHERNOFF, H. M. AND NAHUM, L. H.: Order of recovery from excitation in dog, monkey, and human heart; nature of ST-segment and T-wave. Fed. Proc., Balt., 1949, *8,* 24. From the Laboratory of Physiology.

CHUSID, J. G., GUTIÉRREZ-MAHONEY, C. G. DE, AND ROBINSON, F.: The "motor" cortex of the dog. Fed. Proc., Balt., 1949, *8,* 25. From the Laboratory of Physiology.

DEKKER, C. A., STONE, DAVID, AND FRUTON, J. S.: A peptide from a marine alga. J. Biol. Chem., 1949, *181,* 719. From the Department of Physiological Chemistry.

EPSTEIN, J. A.: Technical notes. A simple multilead needle electrode for intracerebral electroencephalographic recording. Electroencephalog. clin. Neurophysiol., 1949, *1,* 241. From the Laboratory of Physiology.

EPSTEIN, F. H. AND RELMAN, A. S.: Transfusion treatment of shock due to myocardial infarction. N. England J. M., 1949, *241,* 889. From the Department of Internal Medicine.

KAADA, B. R.: Site of action of myanesin (mephenesin, tolserol) in the central nervous system. J. Neurophysiol., 1950, *13,* 89. From the Laboratory of Physiology.

LAMPORT, HAROLD: The law relating blood to perfusion pressure. Fed. Proc., Balt., 1949, *8,* 90. From the Laboratory of Physiology.

LAWRASON, F. D. AND CRONKITE, E. P.: Incidental finding of megaloblastic-like cells in bone marrow of one of two swine with macrocytic anemia and achlorhydria. Yale J. Biol., 1949, *22,* 57. From the Department of Internal Medicine.

LINDSKOG, G. E., LIEBOW, A. A., AND HALES, M. R., Bilobectomy—Surgical and anatomical considerations in resection of right, middle, and lower lobes through the intermediate bronchus. J. Thorac. Surg., 1949, *18,* 616. From the Thoracic Study Group, Departments of Surgery and Pathology.

LIVINGSTON, R. B.: Réponses de la langue et des lèvres à la stimulations du cortex moteur chez le singe (Macaca mulatta). Helv. physiol. Acta, 1949, *7,* 15. From the Laboratory of Physiology.

MILSTONE, J. H.: The chain reaction of the blood clotting mechanism in relation to the theory of hemostasis and thrombosis. Blood, 1949, *4,* 1290. From the Department of Pathology.

NAHUM, L. H. AND CHERNOFF, H. M.: The spread of excitation and the order of depolarization in the human heart. Fed. Proc., Balt., 1949, *8,* 796. From the Laboratory of Physiology.

PETERS, J. P.: Diagnostic significance of electrolyte disturbances. Bull. N. Y. Acad. M., 1949, *25,* 749. Paper read before the Graduate Fortnight of the New York Academy of Medicine.

PETERS, J. P.: The regulation of the volume and composition of body fluids. J. Missouri M. Ass., 1950, *47,* 9. John Auer lecture delivered at St. Louis, Oct. 28, 1949.

PETERS, J. P., MAN, E. B., KYDD, D. M., ENGSTROM, W. W., AND WATERS, L. L.: Toxic effects of antithyroid drugs. Yale J. Biol., 1949, *22,* 139. From the Department of Internal Medicine.

POWERS, G. F.: Remarks to undergraduate students of clinical medicine. Yale J. Biol., 1949, *22,* 109. From the Department of Pediatrics.

ROBERTS, SIDNEY AND WHITE, ABRAHAM: Biochemical characterization of lymphoid tissue proteins. J. Biol. Chem., 1949, *178,* 151. From the Department of Physiological Chemistry.

ROBINTON, E. D.: The effect of staphlococcal enterotoxin upon the frog. Proc. Soc. Exp. Biol., N. Y., 1949, *72,* 265. From the Department of Hygiene and Bacteriology, Smith College, and the Department of Public Health, Yale University.

SCIARINI, L. J. AND FRUTON, J. S.: Derivatives of N-phosphorylated amino acids. J. Am. Chem. Soc., 1949, *71,* 2940. From the Department of Physiological Chemistry.

SHERLOCK, SHEILA: Comparison of the carbohydrate effects of adrenalin infused into the femoral vein, carotid artery, aorta, and portal vein of rats. Amer. J. Physiol., 1949, *157,* 52. From the Department of Physiological Chemistry and the Laboratory of Physiology.

SHRIGLEY, E. W. AND MACULLA, E. S.: Characterization of the agglutinating principle in chorioallantoic fluid responsible for the clumping of certain strains of Staphlococcus aureus. J. Bact., Balt., 1949, *58,* 791. From the Departments of Pathology and Bacteriology.

SIMMONDS, SOFIA AND FRUTON, J. S.: A micro-organism exhibiting a growth requirement for peptides. Science, 1949, *109,* 561. From the Departments of Biology and Physiological Chemistry.

STRAUSS, M. J., SHAW, E. W., BUNTING, HENRY, AND MELNICK, J. L.: "Crystalline" virus-like particles from skin papillomas characterized by intranuclear inclusion bodies. Proc. Soc. Exp. Biol., N. Y., 1949, *72,* 46. From the Division of Dermatology, the Section of Preventive Medicine, and the Department of Pathology.

SZEGO, C. M. AND ROBERTS, SIDNEY: The influence of ovariectomy on the chemical composition of regenerating rat liver. J. Biol. Chem., 1949, *178,* 827. From the Department of Physiological Chemistry.

TALLAN, H. H., CLIFFTON, E. E., AND DOWNIE, G. R.: Spectrophotometric method for assay of serum antiprotease: Clinical applications. Proc. Soc. Exp. Biol., N. Y., 1949, *70,* 667. From the Department of Surgery.

WAGNER, R. R.: Nonspecific inhibition of the lecithinase activity of type A Clostridium welchii toxin. J. Bact., Balt., 1949, *58,* 491. From the Department of Internal Medicine. Work done at the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

WAGNER, R. R., BENNETT, I. L., AND LE QUIRE, V. S.: The production of fever by influenzal viruses. I. Factors influencing the febrile response to single injections of virus. II. Tolerance in rabbits to the pyrogenic effect of influenzal viruses. J. Exp. M., 1949, *90,* 321, 335. From the Department of Internal Medicine; work done at the Naval Research Institute, Bethesda, Md.

WERNER, A. Y. AND GELFAN, SAMUEL: Blood pressure changes following explosive decompression to extreme altitudes. Fed. Proc., Balt., 1949, *8,* 163. From the Laboratory of Physiology.

WILSON, C. C.: Looking ahead in public health. Mississippi Doctor, 1949, *26,* 256. From the Department of Public Health.

BOOK REVIEWS

AVIATION MEDICINE IN ITS PREVENTIVE ASPECTS. An Historical Survey. By John F. Fulton. New York, Oxford University Press, 1948. 174 pp., illus. $3.00.

It seems proper that a physiologist should have written a book about aviation medicine, dealing as it does with I. Altitude Sickness and Acclimatization: The History of Oxygen; II. Decompression Sickness: The Genesis of the Tissue Bubble; III. Pressure Cabins and Explosive Decompression; The Spring of the Air; IV. Effects of Acceleration: Dim-Out and Black-Out; Protective Measures; and V. Man and the Machine: Problems of Safety in Flight.

If you enjoy reading for pleasure, open Dr. Fulton's book at any page and start in. Well-written and readable, it blends history and science in a way which might serve as an example of excellence to many of our medical writers. You will quickly discover that the pilot or the passenger in an aircraft can take to the air and land safely only if the designer as well as the pilot do their work within the limitations of man's physiology.

You will find that the pioneer in this field was a man who never saw an airplane. Robert Boyle wrote *The Spring of the Air* about three hundred years ago, discussed altitude sickness, and first mentioned the occurrence of tissue bubbles in animals confined in low pressure chambers.

Since Boyle's time, the practical applications of his and subsequent physiological observations have become apparent. One might conclude with Dr. Fulton that one of the first things to consider in developing a new engineering discovery would be the way in which the human organism might be affected by the physical and physiological forces involved. Thus, designers should logically ask for consultation from physiologists and other medical scientists early in their labors. That this was not always so is attested to by Dr. Fulton's report of ". . . Air Marshal Harris's expostulations in 1940-1 over the size of the tailgunner's compartment in our early B-17 bombers—compartments which to man would require the development of a race of pygmies—. . . [and] . . . his infinite delight on witnessing an aircraft designer stuck and unable to move in attempting to seat himself at the rear-gunner's station."

This special medical field in which the environmental factors have a vital bearing upon the health of people who fly has expanded largely through the application of sound scientific principles to the problems at hand. This lesson must be applied to every occupational field if we are to succeed in teaching man to live safely and in good health in his industrialized world.

J. WISTER MEIGS

AUDIOLOGY. By Norton Canfield. Springfield, Ill., Charles C Thomas, 1949. ix + 45 pp. $1.75.

This small volume presents the basic principles of audiology in a precise

and orderly fashion. Its thirty-eight pages and excellent bibliography were not written as textbook material but rather as answers to the questions, "What is Audiology?" and "Why?"

Dr. Canfield defines audiology by discussing its component special fields of endeavor and justifies it as a developing professional speciality on the basis of results obtained in the military and Veterans Administration programs. Throughout, it emits a fervent plea for the organization of diagnostic, therapeutic, and rehabilitative measures into a system of therapy for those with defective hearing.

EDWARD H. TRUEX, JR.

COAGULATION, THROMBOSIS, AND DICUMAROL. By Shepard Shapiro and Murray Weiner. New York, Brooklyn Medical Press, 1949. xi + 131 pp. $5.50.

This book is written primarily for clinicians and clinical pathologists who are faced with the practical problems of dicumarol administration. It is well established that dicumarol lowers mortality from thromboembolic disease, but the effects of the drug are delayed, cumulative, and prolonged, and there is much variation in the response of different individuals. Therefore, the drug must be administered with knowledge of the dicumarol effect and with careful laboratory control in each case. Then, the desired therapeutic effect . . . be achieved with minimal danger of hemorrhage.

The difficulty of gauging from any *in vitro* test the tendency to thrombose or the tendency to bleed is fully appreciated by the authors. Moreover, they emphasize that the one-stage "prothrombin-time" does not measure percentage of prothrombin. Nevertheless, experience has shown the "prothrombin-time" to be a useful indicator of dicumarol effect.

In dealing with blood coagulation theory, the authors are in the position of impartial reviewers; and their discussion could hardly avoid the divergent concepts now prevailing. On the practical side, Shapiro and Weiner are leaders in their field. The Link-Shapiro modification of the Quick test has found wide use. Their extensive clinical studies, along with the experiences of others, are briefly and lucidly summarized. There is an interesting chapter on dicumarol levels in plasma and an appendix of laboratory procedures.

J. H. MILSTONE

A YEAR WITH OSLER, 1896-1897. NOTES TAKEN AT HIS CLINICS IN THE JOHNS HOPKINS HOSPITAL. By Joseph H. Pratt. Baltimore, The Johns Hopkins Press, 1949. xx + 209 pp. $4.00.

July 12, 1949 marked the centennial of the birth of Sir William Osler, and demonstrations all over the world of affectionate remembrance have clearly shown that he still exerts a profound influence on his profession. Of the many forms that the tributes have taken, probably none would have pleased Sir William more than the publication of notes from one of his early clinical lecture courses at the Johns Hopkins Hospital. Carefully prepared, with due attention to historical backgrounds and current refer-

ences, and delivered with sufficient deliberation to make note-taking easy, these lectures convey with extraordinary vividness an idea of the actual clinical demonstrations, and incidentally the fidelity with which Dr. Pratt took the notes and subsequently edited them.

The number of cases of typhoid fever and pneumonia seen in the clinics serves to recall the prevalence of those diseases before the turn of the century, and in the case of the former will furnish medical students of a generation that rarely finds typhoid fever on the wards with most of the symptomatology necessary for a correct diagnosis of the disease.

Dr. Pratt's introduction gives a clear, sympathetic vignette of Osler and should lead many students to seek out the formal biography of Cushing for more information concerning that dynamic Canadian, whose spirit hovers benevolently over the profession which is so much richer for the devotion he gave to it.

MADELINE STANTON

A New Theory of Human Evolution. By Sir Arthur Keith. New York, Philosophical Library, 1949. x + 451 pp. $4.75.

In this series of essays finished in his eighty-second year, Sir Arthur Keith not only looks back on a lifetime of patient, thoughtful study, but courageously expounds a theory—if not a new theory, at least "Darwin's theory extended, modified, and brought up to date." It is Sir Arthur's thesis that from earliest times man, like the apes before him, has been a tribal, territorial, competitive animal governed by a dual code of conduct, his behavior toward members of his own group being quite different from that toward strangers. As a result, at least until very recently in human history, mankind has been divided into small, local populations between which there has been relatively little exchange of genes, and thus man's own nature has been a factor in his evolution, accounting on the one hand for its rapidity and on the other for its peculiar character. To the support of this "group theory" Sir Arthur brings evidence from an enormous reading in vertebrate zoology, comparative animal behavior, psychology, and cultural anthropology, as well as from his special field of physical anthropology.

With much of the book the biologist and anthropologist can have no quarrel; it is an admirable exposition of certain aspects of human behavior, ornamented by literary allusions and containing much matter ordinarily avoided by scientific writers, such as "mortality as a factor in human evolution." Sir Arthur's use of the "incendiary term," *race* to include any potential evolutionary unit instead of the distinguishable product of infraspecific evolution, will not be popular (for it restores Jewry to racial status), but at least he has defined his position with care. Unfortunately, he has not made altogether clear the differences, if any, between the evolutionary pattern among mankind and among other higher vertebrates. There is now wide, though not universal, agreement that modern man has arisen polyphyletically, from ancestors belonging to several different species, but polyphylesis in itself is not peculiar to human evolution, nor is "foetalization," to which another chapter is devoted. To make himself fully under-

stood by the technically trained reader in matters like these, the author would have had to expand considerably his middle chapters on the mechanism of evolution; but they are already difficult enough for the general reader, who has no illustrations to guide him.

E. S. DEEVEY

PRACTICAL ANATOMY. By W. E. Le Gros Clark. Baltimore, The Williams & Wilkins Company, 1949. 473 pp. $6.00.

The second edition of this dissecting manual remains unaltered in scope. Revisions, additions, and the use of color have rendered its illustrations one of the main features of this new edition.

The order of dissection is slightly different from that which is often adopted; however, the subdivision of each course of dissection into a series of separate sections facilitates alteration to suit local requirements.

Hints on the art of dissecting contained in the introduction are poignant. Subjects for preliminary and supplementary study are indicated at frequent intervals for amplification of the dissecting-laboratory studies. Deliverance from the awkward and oftimes burdensome descriptive language used in textbooks of anatomy is admirably achieved.

V. L. S.

STUDIES ON HOOKWORM DISEASE IN SZECHWAN PROVINCE, WEST CHINA. By K. Chang and Co-Workers. Baltimore, The Johns Hopkins Press, 1949. 152 pp. $3.00.

Hookworm infestation is widespread in Szechwan, but heavy infections leading to marked impairment of health are concentrated in the hilly regions. This is curious at first sight, since human feces, the source of the infection, are used as a fertilizer throughout the province. It is in these hilly regions, however, that agricultural practice is such as to provide excellent conditions for infection. Corn is planted in the late spring and heavily manured with "night soil," usually several times. Then, two to four weeks later, after a heavy rain, sweet potato vines are set out by hand between the rows of corn. The weather is warm, the fields muddy, the farmers barefoot and barehanded—all conditions favorable to the development and activity of larvae and their intimate contact with the skin. As a result, from 75-99.5% of the population is infected in such corn-sweet potato districts, with average egg counts of 3,000 or more per cc. of feces and frequent clinical cases of "hookworm disease."

The reader cannot fail to be struck by the relativity of the concept "healthy." In the areas of Szechwan investigated, hemoglobin values for "normal" individuals were 50-60% (Sahli Hellige); again, in other regions where only 65 to 75% of the population was infected with hookworm, the infestation was considered of minor importance and scarcely a problem at present.

There is much clinical and epidemiological data which cannot be adequately summarized. The authors are to be doubly congratulated: first, on completing a survey which will probably become a standard for the area

and for suggesting a simple method for control; and secondly, for launching and completing this work in what must have been the very unfavorable war years 1940-44.

DAVID WEINMAN

PSYCHODIAGNOSIS: AN INTRODUCTION TO TESTS IN THE CLINICAL PRACTICE OF PSYCHODYNAMICS. By Saul Rosenweig (with Kate L. Kogan). New York, Grune & Stratton, 1949. xii + 380 pp. $5.00.

This text was designed to provide for "beginning students of professional psychology, as well as . . . those in the allied fields of medicine, nursing, occupational therapy, and social work . . . an up-to-date introduction to psychodiagnosis that could be read in a short time and understood without extensive background." This objective is very well met.

Following an introductory discussion of the role of the clinical psychologist, 155 pages are devoted to descriptions of available materials and how they are used. For each test described, the actual records obtained from a variety of cases are individually presented and interpreted. Several of each of the following are described: tests of intelligence, measures of intellectual impairment, vocational and aptitude tests, personality questionnaires, and the projective methods.

In a separate section, Part III, the author demonstrates how a comprehensive picture of the various aspects of an individual's functioning is obtained by integrating the findings from the data available from previously described tests. In Part IV a variety of case material is presented. This further illustrates how each of the procedures is specifically useful in one or more areas and, at the same time, how it contributes to an understanding of general functioning. How the particular battery of tests applicable in any one case is determined by the presenting problem, by the need for additional information in an obscure area, or by the need for confirmatory evidence for a tentative hypothesis is illustrated in the case materials presented.

The brief closing section of the book deals with "Psychodiagnosis as an Art." Here the author, who views both psychodiagnosis and psychotherapy as "distinctly matters of art as much as of science," takes issue with the assumption that understanding of the individual will in any way be aided by an understanding of the general laws of behavior and predicates the liaison of the clinician and the academic psychologist upon the reorientation of the latter from his emphasis on behavior in the abstract to a study of the individual as the unit and focus of investigation. While earlier interests of the "academic psychologist" have done much to foster a view such as that expressed by Rosenweig, more recent systematic behavior interests and studies yield many hypotheses applicable to the understanding of psychodynamics, and thus, of the individual.

While such errors of omission and commission as are found in the book may lead the uninitiated reader to some erroneous conclusions, these errors are minor compared to the positive contributions contained in the material presented. For the audience to whom the book is directed, it has much to offer.

ANNE M. RITTER